

Q. 610.5
JH
Cap 2

REMOTE STORAGE

THE LIBRARY OF THE
DEC 31 1943
UNIVERSITY OF ALABAMA

BULLETIN

OF

THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

(SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY)

Vol. XXXIV—No. 387]

BALTIMORE, MAY, 1923

[Price, 50 Cents

CONTENTS

PAGE	PAGE
Carcinoma of the Cervix Uteri. A Pathological and Clinical Study With Particular Reference to the Relative Malignancy of the Neoplastic Process as Indicated by the Predominant Type of Cancer Cell. (Illustrated.) By KARL H. MARTZLOFF, M. D. 141	The Blood Picture of Uncomplicated Pellagra. With A Review of the Literature. By JOHN G. HUCK, M. D. 157
Quantitative Studies With Arsphenamine. I. A Colorimetric Method for the Estimation of Arsphenamine in Blood and Tissues. By A. C. KOLLS and J. B. YOUMANS 149	An Instrument for Measuring Distortion Due to the Divergence of X-Rays. (Illustrated.) By EBEN C. HILL 164
A Clinical Study of Ectopic Pregnancy. By LEO BRADY, M. D. 152	The Production of Kidney Lesions in Rats by Diets Defective Only in That They Contained Excessive Amounts of Proteins. (Illustrated.) By L. M. POLVOGT 168
Concerning the Antiseptic Action of Some Benzyl Compounds. By DAVID I. MACHT and JUSTINA H. HILL . . . 154	Notes on New Books 172

Entered as Second-Class Matter at the Baltimore, Maryland, Postoffice.
Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 3, 1918.

CARCINOMA OF THE CERVIX UTERI *

A PATHOLOGICAL AND CLINICAL STUDY WITH PARTICULAR REFERENCE TO THE RELATIVE MALIGNANCY OF THE NEOPLASTIC PROCESS AS INDICATED BY THE PREDOMINANT TYPE OF CANCER CELL

By KARL H. MARTZLOFF, M. D.

(From the Department of Gynecology of the Johns Hopkins Hospital and University)

Our hypothesis, when this study was begun, was that if basal cell cancer with its relatively low grade of malignancy exists on epidermal surfaces of the body elsewhere, why should it not occur in the cervix uteri and warrant an equally favorable operative prognosis? We also felt that the epidermal integument of the cervix differed morphologically in no essential way from skin except for the absence of a stratum corneum, dermis, dermal glands and dermal appendages. In short, why should epidermoid cancer of the cervix differ from epidermoid cancer occurring in other situations?

The object of this study, therefore, was to discover, if possible, whether in this large and common group of epidermoid or squamous cell carcinoma of the cervix the histo-morphology of the predominant types of cancer cells might signify anything relating to the malignancy of the disease. Because of the relatively poor prognosis as to an eventual operative cure which can be given in these cases, it was thought that any information such an investigation might afford would be of definite value.

Our data have been gathered from the records of 387 patients in the wards of the Johns Hopkins Hospital prior to the year 1920. This figure does not represent the total number of cases of carcinoma of the cervix

* Presented before the Medical Society of the Johns Hopkins Hospital, March 5, 1923.

treated in the hospital up to 1920. It includes only those cases of which we have *complete records together with all pathological material*. Of this number the following table (Table I) shows graphically the number traced and their distribution as to operability. The term operability is used here to indicate whether or not the patients were considered to be within the scope of a possible operative cure and were therefore subjected to an operative procedure. The figures in Table I do not, therefore, give a true index of the actual operability of the cases in this study.

TABLE I.

Showing the number of patients operated upon and traced in this series.

Total No. operated upon. 290.	Total No. inoperable. 97.
Operated upon but not traced 87.	Inoperable cases not traced. 43.
Operated upon and traced 203 or 70.2%	Inoperable cases traced 54 or 55.1%
Total number traced—257 or 66.3% of 387 cases	

PLAN OF STUDY

Only cases of definite cancer were studied. Patients that were operated on for cancer in the past years and who on restudy did not show unquestionable cancer have been omitted even though many of them are living and well. We do not feel that we are able to identify so-called *pre-cancerous lesions* and none of the cases in this series can be classed in this group or in a group which might be designated as *questionable early cases*. The cases of early cancer all show a definite carcinomatous invasion none of them apparently as early as some other reported cases.^{1,2}

In order to avoid any bias or pre-conceived clinical impression which might unwittingly influence our attitude, the pathological tissues from the entire series were first studied. Following this the clinical phase of each case was analyzed and an attempt made to locate the patient and her family physician in order to ascertain the ultimate result.

Inoperable cases, or cases in which no operation was performed, were studied only when the sections of tumor which were used for diagnosis were available.

Operable cases: In this group blocks were made from the entire length and antero-posterior thickness of uterus, cervix and vaginal cuff (Fig. 1, A. A.) when the two latter were not entirely destroyed by cancer. The lateral extension of the process was studied by making blocks of the entire peri-cervical area including a wide margin of cervical tissue proper. These blocks were cut at right angles to the longitudinal axis of the cervix as indicated in Figure 1 (B.B.)

Numerous sections were taken from each block but no attempt to study serial sections was made, because of the expense and the interminableness of such a task. Blocks and sections were also made from the tubes and

ovaries when they were removed, which was practically always the case.

The only cases in this study from which we had no gross material in our laboratory was a group reported by Dr. John A. Sampson³ during the years 1902-1905; sections, however, were studied.

The results of twenty-four autopsies are also included in this study, ten of the autopsies being done in inoperable cases and fourteen autopsies on patients previously operated upon.

CELL TYPES STUDIED

In addition to the epidermoid or squamous cell carcinomas, twenty-one cases of adenocarcinoma of the cervix were studied in order to bring our mortality statistics up to date. The cell types were not studied in the adenocarcinomas.

Almost at the beginning it was seen that the morphology of the cancer cells in the epidermoid variety, for the purposes of description, could be arranged into three groups. The designatory terms used in referring to the cell types which represent one or the other of the three groups are merely terms of description and are meant in no way to complicate or modify present anatomical or pathological nomenclature. The cell types representing the three groups of cancer cells encountered have, therefore, been designated as:

The Spinal Type of Cancer Cell.—In this variety the cancer cells (Figs. 3, 4, 5, 6) so designated are morphologically similar to the cells seen in the upper portion of the stratum mucosum of ordinary cervical epithelium as shown in Figure 2a. These cells are usually polyhedral in shape with well defined cell outlines. The nuclei are fairly large, take only a moderately intense hematoxylin stain, frequently have nucleoli and are separated rather widely from each other by an abundant quantity of surrounding cytoplasm which takes a rather pale eosin stain.

The Transitional Type of Cancer Cell, by far the most common, corresponds most nearly in appearance to a well defined layer of cells (Fig. 2b) seen in normal cervical epithelium, which is limited above by the spinal cell layer just described and below by the distinct malpighian layer or stratum germinativum. Because of their resemblance to this intermediate group of cells we are designating them as cells of the transitional type. The cells in this group (Figs. 7, 8) have a very faint or indefinable cell membrane. Their nuclei take a deep blue hematoxylin stain, frequently have nucleoli and are rather closely placed, being separated by a deep eosin-staining cytoplasm much less in quantity and more deeply staining than that separating the nuclei of the spinal cell group. These cancer cells correspond most probably to the variety which Krompecher⁴ refers to as round cell cancer and which he regards as a variety of basal cell cancer.

The Fat Spindle Type of Cancer Cell, (Figs. 9, 10) is not uncommon in combination with other forms and is, as the term indicates, a broad, spindle-shaped cell. The growth would probably be termed "basal cell cancer" by many observers; in fact some of the cancers in this study composed of this type of cell have been designated as basal cell cancers by certain excellent pathologists, and Krompecher⁴ unqualifiedly traces their origin to the stratum germinativum and to the cylindrical epithelium of glandular structures. Others, (MacCallum)⁵ however, are not so willing to ascribe the origin of various histological types of epidermoid cancer cells to morphologically similar layers of the epidermis. Indeed it would seem almost futile to prove, by post-mortem or post-operative histological methods on fixed tissue, the histogenesis of epidermoid cancer from any particular layer of the epidermis. It is not our purpose in this paper to imply in any way the histogenesis of any particular group of cancers from any particular layer of the cervical epithelium. What we wish to do is to call attention to the histo-morphology of the cervical epidermoid cancers and the resemblance existing between them and morphologically similar cells in the normal cervical epidermis.

The term "basal cell" is therefore purposely not used in this study, because it immediately suggests an origin from the stratum germinativum and an idea of benignity which these tumors do not possess when situated in the cervix uteri.

For this reason the term *fat spindle cell* has been used to designate these long and comparatively broad cells which have markedly hematoxylinophilic nuclei, sometimes containing nucleoli, rather closely packed and separated by only a small quantity of eosin-staining cytoplasm. However, the cytoplasm surrounding the nucleus in this type of cancer appears to be more abundant than that seen in basal cell carcinomata of the skin of the face with which it has been compared.

A *scirrhous-like carcinoma* was observed several times appearing as isolated slender cancer cells ramifying between strands of dense, fibrous stroma resembling very closely scirrhous cancer seen in other situations. This group after being studied separately was found to resemble the transitional cell type of cancer so closely in malignancy that it has been included in the latter group.

Incidence of the various cancer cell types.—As this study progressed it was seen that, excluding adeno-carcinoma of the cervix, twelve groups, representing various combinations of the three different cancer cell types, presented themselves. For example, in the group in which the spinal cell type was predominant there occurred, in addition to the pure spinal cell cancer, combinations of spinal and transitional cells as well as combinations of spinal, transitional and fat spindle cells. In the group of cases in which the transitional cell was predominant,

various combinations occurred, as shown in Table II. A number of cases occurred in the transitional and spinal cell combination, in which the proportion of the two types of cells was about equal and these cases are included under the group of transitional and spinal cell cancer in which the transitional cell is predominant.

The group in which the *fat spindle cells* are predominant is a small group and originally had four divisions which in Table II have all been grouped under two headings.

TABLE II.
To show the various cell types of cancer and their different combinations as noted in this study.
The occurrence of epithelial pearls is also tabulated.

Predominant cell type	Combinations of cell types	Incidence per cent.		Occurrence of epithelial pearls	
		No.		No.	Incidence per cent.
SPINAL CELL	Spinal and Transitional	39	10.1%	18	46% of 39.
	Spinal	14	3.6%	8	57.1% of 14.
	Spinal, Transitional and Fat spindle	7	1.8%	5	71% of 7.
	Total	50	15.5%	31	51.6%*
TRANSITIONAL	Transitional and Fat spindle	63	16.3%	4	6.3% of 63.
	Transitional and Spinal	83	21.4%	25	30.1% of 83.
	Transitional Fat spindle, and Spinal	30	7.7%	10	33.3% of 30.
	Transitional	83	21.4%	6	7.2% of 83.
FAT SPINDLE	Total	259	66.8%	45	17.3%*
	Fat spindle and Transitional	29	7.4%	1	3.4% of 29.
	Fat Spindle	18	4.6%	3	16.6% of 18.
ADENOCARCINOMA	Total	47	12.0%	4	8.5%*
	Adenocarcinoma	21	5.4%	0	0

* Of this group had epithelial pearls.

In Table II the percentage incidence of carcinoma cases of the various cell types is recorded together with the percentage frequency with which epithelial pearls were observed in each particular group. In brief, the group of cases in which the cancer is predominately of the spinal cell type forms 15.5% of the entire series and of these 51.6% have epithelial pearls.

The group in which the transitional cell type is predominant form 66.8% and the fat spindle cell group forms 12%, together totalling 78.8% of the entire series. Epithelial pearls were observed in 17.3% and 8.5%, respectively, of the cases in these two groups.

In the following discussion and tabulation the various groupings of the cancer cell types are gathered under four main headings, each heading referring to the pre-

TABLE III.

Showing the percentage incidence of patients operated upon or autopsied in whom the entire length or thickness of the cervix was involved in cancer; and the disease level (duration of symptoms) when this occurred in the various types of cancer.

Predominant cancer cell type		DISEASE LEVEL (DURATION OF SYMPTOMS)																														
		1 mo.		2 mos.		3 mos.		4 mos.		5 mos.		6 mos.		7 mos.		8 mos.		9 mos.		10 mos.		11 mos.		12 mos.		1½ yr.		2 yr.				
TRAN- SITIONAL	% incidence entire length involved	3	LL	16		22	L *	11	L	7		L	13	LLL *	6		7		6		3			0		16		6		6		
		12.5%		69.5%		57.8%		45.8%		58.3%			65%		100%		87.5%		66.6%		100%				86.8%		85.7%		85.7%			
Total No. 200.	% incidence entire width involved	5		15		18		14		6		L	14	LLL *	6		6		7		3			0		14		6		6		
		20.8%		65.2%		47.3%		58.3%		50%			70%		100%		75%		77.7%		100%				77.7%		85.7%		85.7%			
FAT SPINDLE	% incidence entire length involved	2		1		4		2		2	L *	2			2		3			0		0	0		1		7			0		
		66.6%		100%		80%		50%		100%			66.6%		66.6%		75%				0		0			100%		100%				
Total No. 33.	% incidence entire width involved	2			0	3		3		1		3			2		3			0		0	0		1		7			0		
		66.6%				60%		75%		50%			100%		66.6%		75%				0		0			100%		100%				
SPINAL	% incidence entire length involved	1	L *	1	L	3		6	LL*	1		3	L		3	LL	3		1	L	1		1		6		1		2			
		33.3%		50%		50%		66.6%		50%			42.8%		100%		75%		100%		50%		100%		100%		100%		100%			
Total No. 49.	% incidence entire width involved		0		0	3		4	L*	1		4	LLL		2	L	3		1	L		0		1		5		1		2		
						50%		44.4%		50%			57.1%		66.6%		75%		100%			0		100%		83.3%		100%		100%		
ADENO- CARCINOMA	% incidence entire length involved		0		1	L		0		1		1			2		2			0		3	L		0		0		2		2	
						100%		50%		100%			50%		100%		100%			0		75%				0		66.6%		100%		
Total No. 19.	% incidence entire width involved		0		0	0		1		1		2			2		2			0		4	L		0		0		3	L *	2	
								50%		100%			100%		100%		100%					100%						100%		100%		

KEY: Percentage figures indicate the percentage of the total number of cases met with that occurred during that particular period, viz.:

3 indicates the number of cases during first month of disease with entire length of cervix involved and this is 12.5% of the total number (24) of cases that occurred in this type of cancer with the disease of one month's symptomatic duration.

L or L
L indicates one case living and well to-day. (Cure?)

* indicates one case in which a primary curettage was done several days before radical operation on a patient who is now living and well.

dominant cancer cell type,—Transitional, Fat Spindle, Spinal, and Adeno-carcinoma. In order to determine whether or not differences in morphology indicate a difference in the malignancy of the corresponding types of cancer, it was of course necessary to study the whole subject from the standpoint of the duration of the disease, in order to compare at similar disease levels, so to speak, all the various types of cancer.

In order to estimate the length of time the malignancy had existed in any one case, the time from the appearance of the first definite symptom until the day of the operation, in operative cases, or until the time of diagnosis in non-operated cases, has been taken as our means for measuring the duration of the disease. In the great majority of the cases studied, the first symptoms were quite definite and in most instances supplied a fairly accurate date for the first subjective manifestation of the disease.

We quite fully appreciate that this method is open to objections because of manifest and inherent opportunities for inaccuracies. Under the circumstances, however, it is the only way we have by which to arrive at any estimation of disease duration, and we can only hope that the error so unavoidably entailed may be somewhat attenuated by the fairly large number of cases studied.

LOCAL AND REMOTE MANIFESTATIONS OF THE DISEASE IN RELATION TO CELL TYPE AND DURATION OF DISEASE.

1. *Extent of cervical involvement.*—The malignant process was confined to the pars vaginalis of the cervix in 26.7% of 301 cases of patients operated upon or autopsied. In 8.3% of the cases the cancer occupied about two-thirds of the length of the cervix. In 196 or 65% of the cases the entire length of the cervix was involved, while the entire thickness in one portion or another showed complete involvement by carcinoma in 62.1% of the material studied. In 20.9% of the cases the involvement in thickness was limited to the superficial one-third (involving one-third or less) of the cervix and in the remaining cases the involvement penetrated at least two-thirds of the cervical thickness.

Table III shows the cases in which the entire length or entire thickness of the cervix was implicated in the cancer. The relation between the cell type, in the operated or autopsied cases, and the percentage incidence of complete involvement at various disease levels are recorded.

By consulting Table X in the second part of this paper, the percentage incidence of operated and inoperable cases at different time levels can be seen and compared, in order to secure an idea of how rapidly the malignant process sometimes progresses.

The figures in this table are not particularly relevant in showing decided differences in the rapidity with which cancers of the various cell types involve the cervix. They are, however, astonishing and alarming in that they indicate such an extensive involvement at a relatively early stage of the disease, as estimated by the duration of symptoms.

2. *Location of the Cervical Cancer.*—The situation of the carcinomatous process was often so difficult to determine that in a total of 294 cases that lent themselves for this particular observation no conclusion could be arrived at in 7.8% of the material.

In 27 cases (9.1%) the cancer was of the "inverting type" so that no roughening or ulceration occurred about the external os and no bleeding resulted from manipulation.

In 19 cases (6.46%) the anterior portion and in 16 cases (5.4%) the posterior portion of the cervix was solely and therefore primarily implicated in the cancer.

The anterior, posterior and lateral portions of the cervix were, as well as could be determined, pretty uniformly involved in 195 instances (66.3%).

The carcinoma was limited to the right lateral portion of the cervix in 5 cases (1.07%); to the left lateral portion in 3 instances (1+%); and in one case the right and left halves of the cervix were simultaneously and solely involved in cancer, there being no contiguity between the two processes.

Of the carcinomas which apparently began in a lateral position of the cervix and extended antero-posteriorly to involve only one entire lateral half of the cervix, there were 5 cases (1.07%), two occurring on the right and three on the left half of the cervix.

3. *Associated Involvement of Vaginal Mucosa* was observed in 196 instances* (65.1%) of the patients operated upon or autopsied. Of the 71 cases in which the carcinoma was confined to the pars vaginalis cervicis, the neoplastic process also involved the vagina in 38 cases or 53.5% of the 71 cases. In the cases in which about two-thirds of the length of the cervix was involved 21, or 87.5%, showed associated vaginal involvement; where the entire length of the cervix was involved in cancer, the vaginal mucosa was also affected in 137 instances, or 77.7% of 196.

4. *Associated Involvement of the Corpus Uteri.*—Extension of the cervical cancer to the body of the uterus was observed unmistakably in 81 (26.5%) of the patients operated upon or autopsied. In twelve instances (4%) this involvement was debatable. The uterine involvement was observed only in those cases in which the entire length of the cervix was involved in cancer, so

that of the 196 cases in which the cervix was so altered, uterine extension was observed in 81 cases of 196 (41.3%).

These observations are considerably at variance with those of Hurdon⁶ who states that carcinoma of the cervix uteri rarely invades the body of the uterus and considers the internal os as an upper boundary of the growth.

The extent to which the body of the uterus was involved varied considerably, the involvement of the entire uterus (body and fundus) being observed in only one instance. In sixteen instances the malignant process involved as much as the distal half of the corpus uteri, and in the remaining cases the extension was limited to about the distal one third.

5. *Associated Broad Ligament (Paracervical) Involvement.*—Secondary involvement of the broad ligament either by direct extension or metastasis of the cervical neoplasm was observed in 120 cases. The relation between the thickness of the cervical involvement and the broad ligament involvement is given in Table IV. No broad ligament involvement was noted in the cases where less than one-third of the thickness of the cervix was implicated.

TABLE IV.

Showing the relation of broad ligament involvement to the thickness of cervix involved in cancer, where one third or more of the cervix is so involved.

Thickness of cervix involved in cancer	Number of cases	Number of cases of bd. lig. involvement	Per cent. of cases with bd. lig. involvement
One third to one half	20	3	15% of 20
One half to two thirds	15	3	20% of 15
Entire thickness	189	114	60.3% of 189
Total	224	120	53.6% of 224

Although out of a total of 301 cases 120 (39.1%) showed broad ligament involvement, the latter figure assumes still more important proportions when studied with relation to the 224 cases in which one-third or more of the thickness of the cervix was involved in cancer, the incidence now rising to 53.6%. The particular significance of this is realized when, as will be shown later, no patient in this series with broad ligament involvement was cured by operation.

To compare the incidence of broad ligament involvement in relation to the predominant type of cancer cell and the duration of the disease, Table V was prepared. In this table under each disease level is given the percentage incidence of operated and autopsied cases and also the percentage incidence of broad ligament involvement of these same cases for the corresponding time interval or disease level.

From the figures in Table V it appears that while the percentage incidence of cancer of the various predominant cell types is roughly parallel for the different disease levels, the same cannot be said for the percentage inci-

* This does not include the so frequent inoperable cases in which the vagina is extensively involved and no operation or autopsy is performed.

TABLE V.

Showing under the various predominant types of cancer cells and at the different disease levels the incidence of operated and autopsied cases and the per cent incidence of these having broad ligament involvement.

Predominant cell type		DISEASE LEVEL (DURATION OF SYMPTOMS)													
		1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.	9 mos.	10 mos.	11 mos.	12 mos.	1½ yr.	2 yr.
TRANSITIONAL	% incidence of operated or autop. cases	24 * p3	23 p2	38 p10	24 p *	12 * p.	20 * p2	6 p.	8 p.	9 ‡ p3	4 p.	0	18 p4	7 * p	7 * p2
		12%	11.5%	19%	12%	6%	10%	3%	4%	4.5%	2%	0	9%	3.5%	3.5%
Operated or autop.—200 Cases with epi. pearls—32	% incidence broad ligament involved	2	8	12 p2	10	4	9 p.	3 p.	1	7 p2	3 p.	0	15 p3	5 p.	6 p.
		8.3%	34.7%	31.6%	41.7%	33.3%	45%	50%	12.5%	77.7%	75%	0	83.3%	71.4%	94.2%
FAT SPINDLE	% incidence of operated or autop. cases	3 *	1	5	4 p.	2	3 *	3	4	0	0	0	1	7 p.	0
		9.1%	3.1%	15.1%	12.1%	6%	9.1%	9.1%	12.1%	0	0	0	3+	23+	0
Operated or autop.—33 Cases with epi. pearls—2	% incidence broad ligament involved	2	0	3	0	0	2	1	2	0	0	0	0	7 p.	0
		66.6%	0	50%	0	0	66.6%	33.3%	50%	0	0	0	0	100%	0
SPINAL	% incidence of operated or autop. cases	3 p2	2 p.	6 p3	9 p6	2 p2	7 * p	3 p2	4 p2	1 p.	2	1	6 p3	1	2 p2
		6.1%	4+	12.2%	18.1%	4+	14.2%	6.1%	8.1%	2+	4+	2+	12.2%	2+	4+
Operated or autop.—49 Cases with epi. pearls—26	% incidence broad ligament involved	0	0	1 p.	0	0	1	1	1	0	0	0	3 p.	0	2 p2
		0	0	16.6%	0	0	14.2%	33.3%	25%	0	0	0	50%	0	100%
ADENOCARCINOMA	% incidence of operated cases	0	1 5.3%	0	2 10.5%	1 5.3%	2 10.5%	2 10.5%	2 10.5%	0	4 21%	0	0	3 15.7%	2 10.5%
	% incidence broad ligament involved	0	0	0	1 50%	0	1 50%	2 100%	2 100%	0	2 50%	0	0	1 33.3%	1 50%

KEY: * One inoperable case which was autopsied included in this group.
‡ Two inoperable cases which were autopsied included in this group.
p, p2, etc.=One, two, three, etc., cases with epithelial pearls included in this group.

24Is the total number of cases occurring in this period and is 12% of 200.
12%	
2Is the total number of cases occurring in this period with broad ligament involvement and is 8.3% of 24.
8.3%	

dence of broad ligament involvement. Particularly is this true for the early months of the disease, so that in summarizing the total incidence of operated or autopsied cases and the corresponding broad ligament involvement for the various cell types for the first six months of the disease, the result is as shown in Table VI.

TABLE VI.

Summarizing Table V, for the first six months in order to show the incidence of the cases as they came to this clinic and their corresponding incidence of broad ligament involvement in operated or autopsied cases.

Predominant cell type	Total No. oper. or autop. in first six months	Total No. with bd. lig. invol. in first six month period	Total incidence % of oper. or autop. cases in first six mos.	Total incidence % broad lig. involved in first six mos.
TRANSITIONAL Total number—200	140	45	70%	32.1%
FAT SPINDLE Total number—33	18	7	54.5%	38.8%
SPINAL Total number—49	29	2	59.1%	6.8%
ADENOCARCINOMA Total number—19	6	2	31.5%	33.3%

The outstanding fact in this summarization of cases in patients who came to us during the first six months of their disease is the very low comparative incidence per cent of broad ligament extension in the cancers of the predominant spinal cell type. This small percentage of broad ligament involvement obtains despite the high in-

cidence per cent (59.1%) of all patients with spinal cell cancer. This incidence during the first six months, of patients with the spinal cell type of cancer, is roughly parallel with the incidence of the transitional cell type (70%) and the "fat spindle" cell type (54.5%). However, we find that while in the fat spindle cell type for the first six months the incidence percentage is lowest for the whole group of epidermoid cancers, nevertheless, the frequency of broad ligament involvement is highest (38.8%) in the fat spindle variety of cancer.

The adeno-carcinomas while showing a comparatively low total incidence in Table VI have, on the other hand, a comparatively very high incidence of broad ligament involvement.

In brief, of all the patients, excepting those with the spinal cell type of cancer, who are seen during the first six months of their disease, one-third or more have extension of the malignant process beyond the cervix into the pericervical tissues.

The relation of broad ligament involvement to the duration of life after operation is also interesting. No patient in this series in whom broad ligament involvement was noted is living today, all having died of recurrent carcinoma. The usual duration of life after operation of cancer cases with broad ligament involvement is

considerably less than one year. However, there are exceptions, so that one cannot be too sweeping in offering an early hopeless prognosis in these cases.

In this series twenty patients with broad ligament involvement lived one year or more after operation. Of these ten lived one year and then died of recurrent cancer, half of these belonging to the spinal cell variety of cancer and the remainder to the transitional cell type.

Of the ten remaining patients, three lived two years, the cancer in this group being of the transitional cell type. Three patients lived three years, the cancer types here being adeno-carcinoma, spinal cell cancer and the transitional cell type. One patient in whom the transitional cell type was predominant lived five years after operation, and three with the spinal cell type lived respectively 6, 8, and 12 years after operation, before dying of recurrent cancer.

6. *Associated Rectal Involvement* was noted in 6 cases (2%), and in one case the extension was questionable. In all of these cases the entire thickness of the cervix was involved, so that the neoplastic process had extended evidently by direct contiguity. This probably does not represent the incidence of actual rectal involvement which presumably occurs more frequently in advanced cases which are so often inoperable. However, rectal involvement is at best not a frequent clinical observation even in the hopelessly inoperable cases that we see so frequently.

7. *Associated Involvement of the Urinary Bladder* was observed in 27 cases (9%). Here again the process was evidently one of direct extension, the cervix in each instance being involved throughout the entire anterior thickness.

8. *Metastases to the Fallopian Tubes* were observed in 13 instances (4.3%). In none of these specimens did the metastases involve the tubal mucosa or encroach upon the lumen of the tube. The process was sub-serous and usually involved the musculature and the sub-mucous layer of the tube.

9. *Ovarian Metastases* were noted only in three instances, the metastasizing cancer in all instances being composed of cells predominantly of the transitional type.

10. *Lymph Gland Metastases.* The parametrial glands were involved in 7 cases in which no other glands were available for examination.

In 66 cases in which the regional lymph glands were examined for neoplastic involvement, a secondary carcinomatous extension to the iliac and lumbar glands and in one case to the inguinal glands was observed in 28 cases, and the absence of any such dissemination was demonstrated in the remaining 38 cases, (57.5%) of 66.

11. *Other Metastases.*—The lungs showed metastatic carcinoma in 4 cases: (1). During the fourth month of symptomatic disease in a transitional cell type of cancer

also associated with liver metastases; (2) During the sixth month of symptomatic disease in an inoperable spinal cell tumor where the neoplastic process had also extended to the peritoneal surfaces, to the left ureter and the corresponding kidney; (3). In a case of adeno-carcinoma during the seventh month of symptomatic disease pleural metastases were noted; (4). In an inoperable case of transitional cell type of cancer following a sympto-

TABLE VII.

Showing the various metastases and extensions (except those to lungs, peritoneum and kidneys) of the cervical cancer in relation to the cell types concerned and the disease level at which they were observed.

Predominant cell type	Extensions or metastases	DISEASE LEVEL (DURATION OF SYMPTOMS)														Total No.
		1 mo.	2 mos.	3 mos.	4 mos.	5 mos.	6 mos.	7 mos.	8 mos.	9 mos.	10 mos.	11 mos.	12 mos.	1½ yr.	2 yr.	
TRANSITIONAL	To Uterus. No. of cases.	0	5	8	3	0	4	3	2	2	3	0	7	4	4	45 cases
	To Parametrial glands.	0	1	1	0	1	0	0	1	0	0	0	0	1	0	5 cases
	Iliac glands.	0	*	2	1	0	3	0	0	2	1	0	0	1	1	14 cases
	Lumbar glands.	2	0	1	1	1	0	0	0	0	0	0	0	0	1	6 cases
	Fallopian tubes.	0	0	P 3	*	1	0	0	1	1	0	0	0	0	2	8 cases
	Ovaries.	0	0	1	1	0	0	0	0	0	0	0	0	0	1	3 cases
	Urinary bladder.	1	2	1	1	1	1	0	0	1	1	0	2	1	2	14 cases
FAT SPINOLE	To Uterus.	1	0	0	1	0	1	2	2	0	0	0	1	5	0	13 cases
	To Parametrial glands.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1 case
	Iliac glands.	0	0	1	0	0	1	0	0	0	0	0	0	1	0	3 cases
	Fallopian tubes.	0	0	0	0	0	1	0	1	0	0	0	0	1	0	3 cases
	Urinary bladder.	1	0	0	0	1	1	1	2	0	0	0	1	0	0	7 cases
SPINAL	To Uterus.	0	0	1	4	1	1	1	2	1	0	0	3	0	2	16 cases
	Iliac glands.	0	0	1	1	0	0	0	0	0	0	0	0	1	0	3 cases
	Lumbar glands.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1 case
	Fallopian tubes.	0	0	0	0	0	0	0	P 1	0	0	0	0	0	0	1 case
	Urinary bladder.	0	0	0	0	0	P 1	1	0	0	0	0	P 2	0	0	4 cases
ADENOCARCINOMA	To Uterus.	0	0	0	1	0	2	1	0	2	0	0	0	1	0	7 cases
	To Parametrial glands.	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1 case
	Iliac glands.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1 case
	Lumbar glands.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1 case
	Fallopian tubes.	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1 case
	Urinary bladder.	0	0	0	0	0	0	1	0	0	1	0	0	0	0	2 cases

KEY: * One case. Primary curettage several days before radical operation.
P One case having epithelial pearls.

matic disease duration of twenty-nine months metastases were noted on the pleural and peritoneal surfaces and in the peribronchial lymph glands.

In all of the above cases there was extensive involvement of the cervix and uterus with metastases to the regional lymph glands.

CARCINOMA DEVELOPING IN A RESIDUAL CERVIX AFTER A SUPRAVAGINAL HYSTERECTOMY

Of this particular condition, usually referred to as cancer in the "cervical stump," we had 5 cases, all in women who had borne children;

CASE 1.—The patient, aged 38, had undergone a supravaginal hysterectomy four years before developing cervical cancer. She had complained of vaginal bleeding for eight months prior to admission to this hospital. She died sixteen days after an operation for radical removal of the cervix. Diagnosis: *Cancer of the transitional cell type with some fat spindle and spinal cells.*

CASE 2.—A patient with syphilis, 43 years of age, had undergone a supravaginal hysterectomy 19 years prior to her cervical lesion. One month before entering this hospital she had had constant vaginal bleeding. Operation: Radical removal of the cervix. She is living and well today, 9 years after operation. Diagnosis: *Cancer of the transitional and spinal cell type with epithelial pearls. Cancer limited to superficial portion of pars vaginalis cervicis.*

CASE 3.—A woman, aged 39, had been submitted to a supravaginal hysteromyomectomy five years before the cervical symptoms. Four days before entering the hospital she had begun to have profuse vaginal bleeding. Operation: Diagnostic curettage. Four days later operation for radical removal of cervix. Result: She lived seven years after operation and then died without evidence of local recurrence. Cause of death not known. Diagnosis: *Cancer predominantly of the transitional cell type with some spinal cells and epithelial pearls, limited to superficial third of pars vaginalis cervicis.*

CASE 4.—A woman with syphilis, 38 years of age, had undergone a supravaginal hysterectomy fifteen years before cervical symptoms. Eight months before entering this hospital vaginal bleeding had begun. Operation: Diagnostic curettage. Four days later operation for radical removal of cervix. Well for one year after operation, then lost track of. Diagnosis: *Cancer predominantly of transitional cell type with some fat spindle cells and epithelial pearls. Entire length and thickness of cervix involved.*

CASE 5.—A woman, syphilitic, aged 44, had undergone a supravaginal hysterectomy five years before the cervical symptoms. Four months before entering this hospital she had begun to have vaginal bleeding. Condition: Inoperable cancer predominantly of the *transitional* cell type with some spinal cells. Result: Died two years after leaving hospital.

The incidence of carcinoma in a "cervical stump" is, to say the least, rare judging from these figures which give for the entire series of 387 cases a percentage incidence of 1.3% and a percentage of a five year cure of 40% for these five cases.

CONCOMITANT OR ASSOCIATED LESIONS OF THE PELVIC ORGANS NOT MALIGNANT IN NATURE

1. CERVIX. *Endo-cervicitis* was an almost constant finding not infrequently associated with an accompanying inflammatory process involving the broad ligaments.

Nabothian Follicle Cysts were observed twelve times in 301 cases (3.9%). In a few of these cases the cancer was a fairly well advanced process, in one instance involving the entire length and thickness of the cervix.

2. UTERUS. In 202 cases the endometrium was suitable for examination.

Endometritis. Under this term are included only those cases in which a definite inflammatory process was present. By chronic endometritis we mean at least, in the absence of other signs of inflammation, the presence of round wandering and plasma cells in the interglandular stroma. The mere presence of lymphocytes even in large numbers was not considered indicative of a chronic inflammatory reaction because lymph follicles occur with varying frequency in almost all endometria and the presence of lymphocytes is, therefore, of questionable pathological significance. The cases of tuberculous, acute and sub-acute endometritis, naturally do not offer the same difficulty.

Endometritis was observed in 11 cases (5.4%). In five of these the cancer had extended to the distal one-third of the corpus uteri, in one case such extension was debatable and in the remaining five the body of the uterus was not involved in the neoplasm.

Pyometra. With one exception the presence of pus or of a malodorous and turbid fluid in the uterine cavity was a clinical observation, noted at the time of operation when the cervix was dilated. The one exception was in an inoperable case that came to autopsy. Pyometra was noted 11 times in this series. Two of these cases are included under the heading of endometritis. Of the others some were inoperable, while others from which the uterus was obtained showed a complete absence of endometrium and the presence of an inflammatory cellular infiltration of the myometrium, a true myometritis.

Hyperplasia of the Endometrium was observed in 5 (2.4%) of our series. A condition of the endometrium characterized clinically by menorrhagia and pathologically by irregularly dilated endometrial glands lined with columnar epithelium, surrounded by a dense interglandular stroma in which mitotic figures are often visible, was first described and given its name by Cullen,⁷ and since has been the subject of considerable interest.^{8,9} In this country the condition is frequently and incorrectly referred to as "chronic interstitial or polypoid endometritis."

Endometrial Polypi were observed in 8 cases (3.9%) and were all of the glandular type. The polypi were all single excepting in one case where the uterine cavity contained several. The endometrium in these instances was essentially normal otherwise, no evidence of hyperplasia or endometritis being seen.

Pregnancy.—In two cases (.99%) the endometrium showed an unmistakable decidual reaction. This does

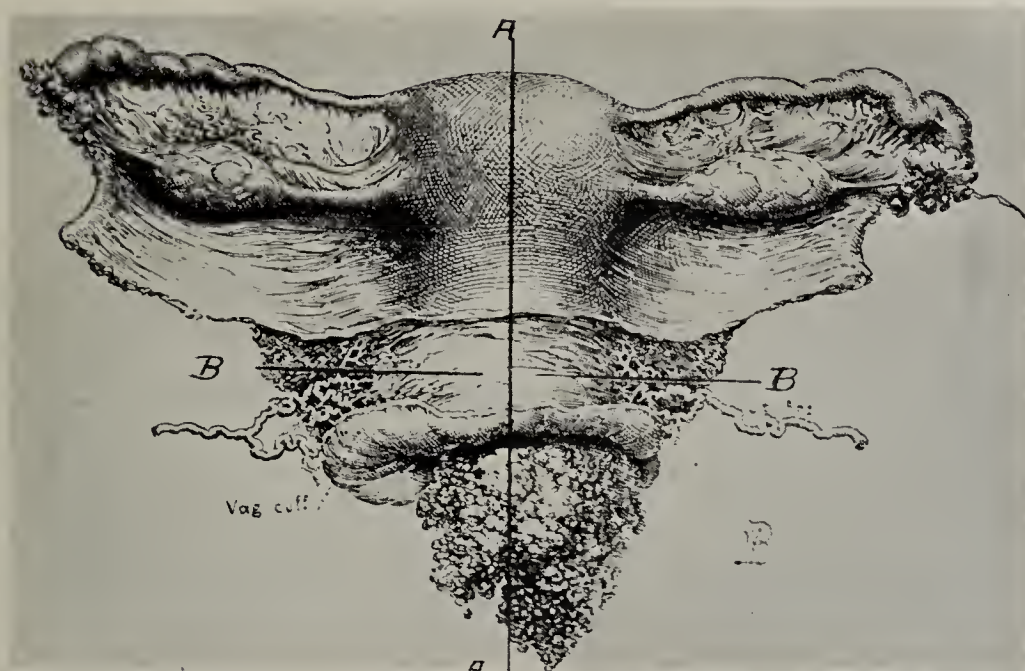


Fig. 1.—A posterior view of a uterus with a cervical carcinoma. The line A.A. indicates the direction in which blocks were cut in order to study the extent of the process both antero-posteriorly and superior-inferiorly. The lines B.B. indicate the direction and approximate depth of the blocks taken throughout the broad ligament regions in order to study the lateral progress of the neoplastic process. (Modified after J. G. Clark.)



Fig. 2.—Normal stratified cervical epithelium. The layer of cells indicated by *a* are the characteristic "prickle or spinal cells" seen in normal epidermis from any situation. As can be distinctly seen, the cells are large, polyhedral, have a distinct cell membrane, and a very pale eosin staining cytoplasm. At *b* the cells in the normal cervical epithelium neither resemble the cells at *a* nor do they resemble the cells of the stratum germinativum. They are, so to speak, cells morphologically of an intermediate or *transitional* type. The stratum germinativum is not indicated here because it is not well brought out in this photomicrograph.



Fig. 3.—(Gyn. Path. No. 12125.) Spinal cell cancer with epithelial pearls.

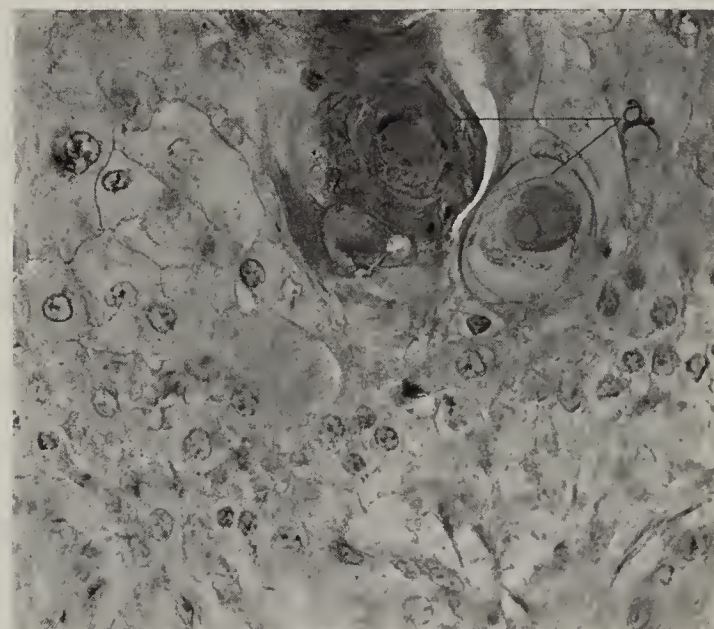


Fig. 4.—(Gyn. Path. No. 12125.) A high power of Figure 3, showing epithelial pearls at *a*. To the left of the epithelial pearls are seen typical spinal cells with well defined cell membrane, polyhedral outline, and large quantity of pale eosin staining cytoplasm.

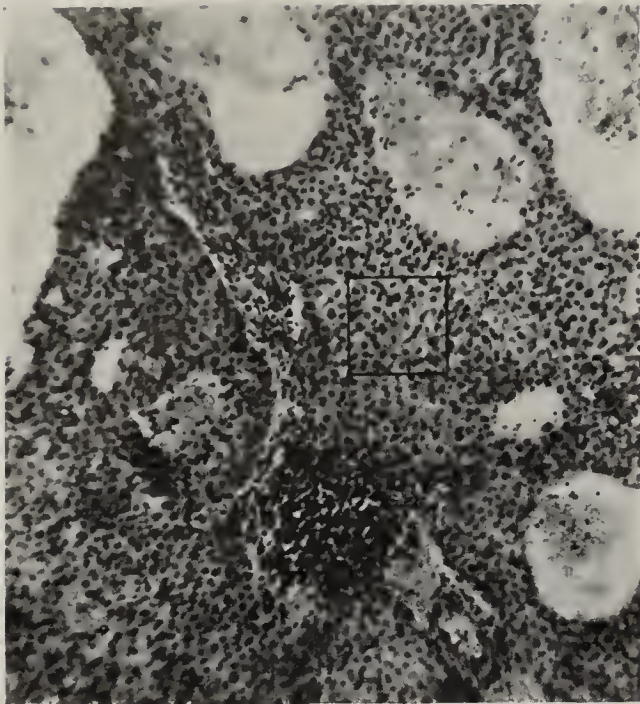


Fig. 5.—(Gyn. Path. No. 26737.) Spinal cell cancer without epithelial pearls.

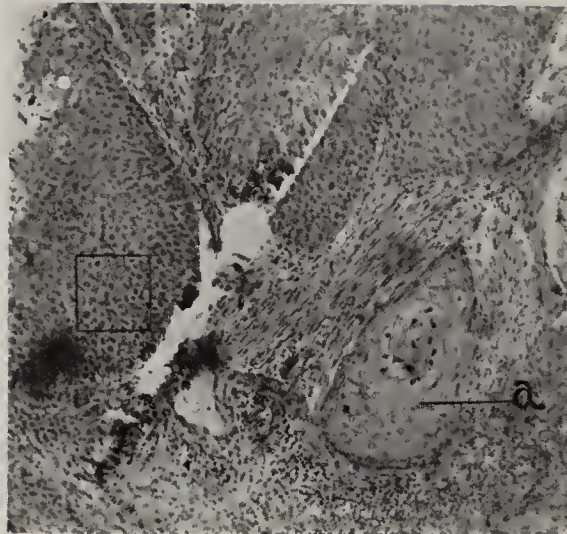


Fig. 7.—(Gyn. Path. No. 13370.) Transitional and spinal cell cancer. The predominant cell type here is transitional. At *a* is an area of cancer where the cells are of the spinal cell type.

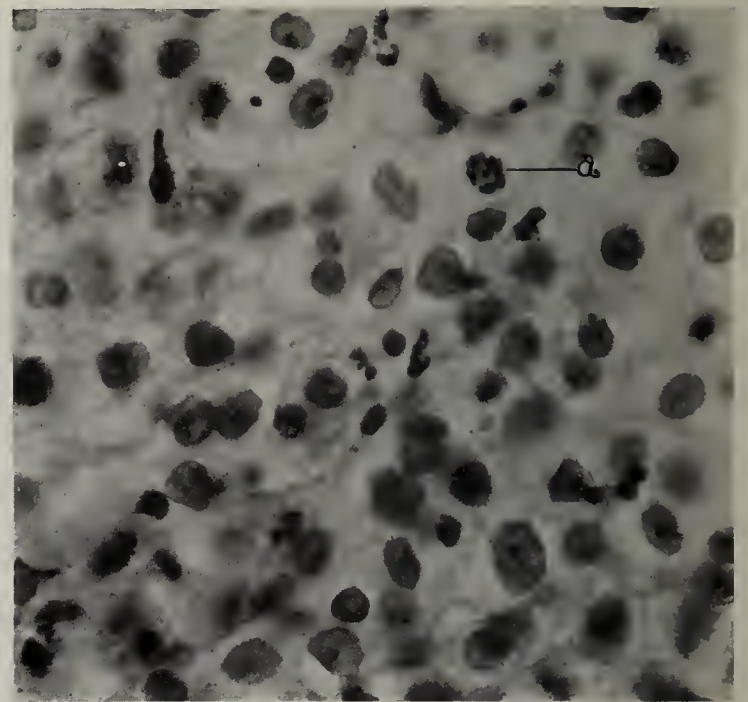


Fig. 6.—(Gyn. Path. No. 26737.) A high power picture of area inclosed in square in Figure 5, showing typical spinal cells with a mitotic figure at *a*.

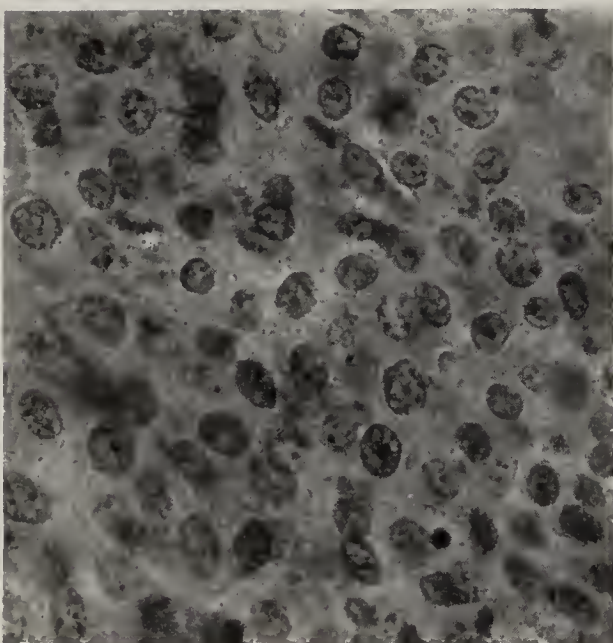


Fig. 8.—(Gyn. Path. No. 13370.) A high power picture of area inclosed in square in Figure 7, showing the cells characteristic of the transitional type of cancer.

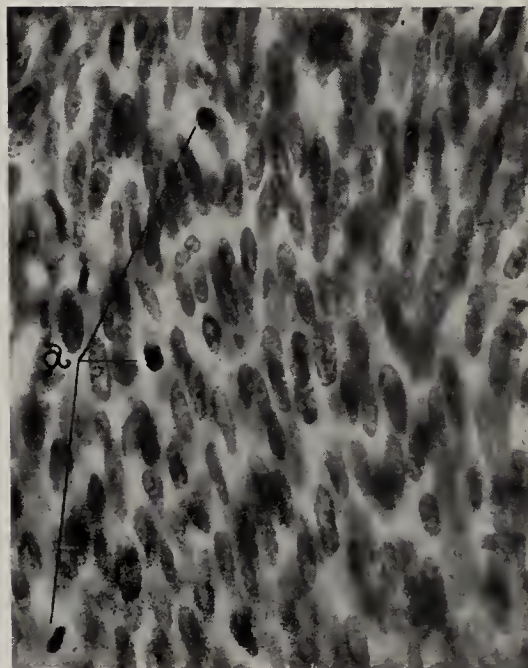


Fig. 10.—(Gyn. Path. No. 22556.) *Fat Spindle cell* cancer from the same specimen as the section shown in Figure 9. This photomicrograph shows even more distinctly the long, broad cells. At *a* are mitotic figures which do not appear very distinct in this picture.

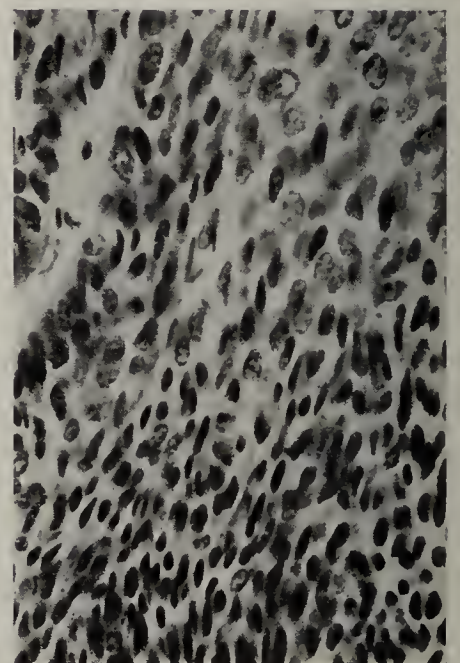


Fig. 9.—(Gyn. Path. No. 22556.) *Fat Spindle cell* cancer showing the cells with their broad and elongated nuclei, closely placed and with little intervening eosin-staining cytoplasm.

not, however, indicate the actual relation in this series of pregnancy and cancer which is noted elsewhere.

Adeno-carcinoma of the Fundus Uteri was observed once in association with an epidermoid cancer of the cervix which had not invaded the body of the uterus. The adeno-carcinoma of the fundus was a totally independent process separated from the cervical neoplasm by normal endometrium and myometrium.

Myomata Uteri.—In 28 instances (9%) uterine myomata were associated with cervical carcinoma. In some of these they were small, single and only an incidental finding. In one case in which the cervical neoplasm had involved the corpus uteri rather extensively, a myoma was found showing definite carcinomatous invasion.

Adeno-myomata Uteri.—This condition so exhaustively described by Cullen¹⁰ was observed in 8 instances. In three of these eight cases the uteri contained myomata, some of which in turn enclosed endometrial glands and interglandular stroma. A diffuse adeno-myomatous condition of the myometrium (adeno-myoma uteri diffusum benignum) was unmistakable four times and in one specimen a diffuse adeno-myomatous tendency was apparent, the endometrial elements invading the myometrium considerably beyond the limits seen in normal uteri.

3. FALLOPIAN TUBES. *Salpingitis* was observed as a simple, chronic inflammatory process 68 times (22.2%).

In 8 cases one or both tubes were distended and filled with pus and in 2 cases there was a hydrosalpinx.

Tuberculous salpingitis was observed in 2 cases, one of these patients who lived two years after operation having a concomitant tuberculous peritonitis. The other patient is living and well today, fourteen years after operation.

Tubo-ovarian abscesses were observed in 3 cases, in all of which they had ruptured, causing a secondary peritonitis. One of these patients recovered, lived four years after operation, and then died of a recurrent adeno-carcinoma.

4. OVARIES.—Primary ovarian pathological conditions in this series were noteworthy by their absence. It should be stated, however, that we did not try to tabulate the presence of corpora lutea, follicular cysts, etc. The only two definite primary ovarian lesions observed were one dermoid cyst and in one case bilateral ovarian papillary cystadenomata.

5. SYPHILIS.—Since 1912, when the Wassermann reaction on every patient's blood serum became a routine procedure, we have in this study 116 cases of cervical cancer (inoperable or operable) of which 8 (6.8%) had a positive Wassermann reaction. One of the luetics is living and well today, 9 years after an operation for carcinoma developing in a cervical stump.

NOTE.—This paper will be concluded in the June Bulletin.

QUANTITATIVE STUDIES WITH ARSPHENAMINE

I. A COLORIMETRIC METHOD FOR THE ESTIMATION OF ARSPHENAMINE IN BLOOD AND TISSUES*

By A. C. KOLLS and J. B. YOUMANS

(From the Department of Medicine, Johns Hopkins University)

Our knowledge of the concentration, distribution, and excretion of arsphenamine depends on data calculated from arsenic determinations which have been made after wet ashing the tissues and body fluids. The assumption that this arsenic was still in the form of the dioxy-diamino-arsenobenzol is not warranted, for it is well known that this substance is oxidized with considerable ease. Furthermore, the appearance of arsenic in a particular tissue is not the best evidence that arsphenamine was deposited there, for it is possible that the original molecule may have undergone considerable change elsewhere in the body with the result that simpler products were liberated in the circulation to appear at some distant point.

The earlier work on the arsenic content of various body fluids has been summarized by Myers,¹ who examined the blood and spinal fluid in a number of cases where the drug had been administered. More recently Clausen and Jeans² investigated the distribution and excretion of

arsenic in children after the injection of arsphenamine. In all this work it is evident that a method which permits study of the fate of the remaining portion of the molecule would aid considerably in an understanding of the behavior of the substance in the body.

Having in mind the use of some part of the arsphenamine molecule other than the arsenic, we tried the qualitative test described by Abelin.³ This test makes use of the fact that the amino groups of arsphenamine are readily diazotized and linked with compounds of the dioxy-benzene series. From the work which has been mentioned it was obvious that any method to be of value must be an extremely delicate one. The diazo reaction fulfilled this requirement, for a distinct color occurs with it in dilutions in excess of 1 in 2,000,000. It remained to be determined, however, whether other substances occurring in the tissues also gave the reaction

* Presented before the Johns Hopkins Hospital Medical Society, March 6, 1922.

and whether the reaction was a quantitative one. Abelin found that normal urine gave negative tests. He anticipated the application of the reaction to a quantitative method but gave no suggestions as to its use in this connection. On testing these factors we were assured that the reaction was a dependable one. It became necessary then to find some deproteinizing agent which would neither interfere with the color reaction nor remove the drug with the coagulable matter.

That the reaction is a quantitative one fairly independent of the concentrations of the reagents has been confirmed by a number of experiments. Table 1 shows the results of one of these tests. The color develops promptly if sufficient alkali is used. It is bright red and is easily matched in the colorimeter. Of the various phenols which might be used, orcin is the least objectionable. Resorcin gives a color which is as brilliant as the one produced with orcin, but the former compound rapidly turns green in alkaline solution. Orcin is also subject to change in the presence of alkali, but the product is pink. With the concentrations of the reagent and alkali employed in our work the increase in the intensity of the color is negligible.

It has been found that the amount of acid, 0.5 cc. of one-tenth normal hydrochloric acid, and the amount of sodium nitrite, 0.5 cc. of a one per cent solution, have no effect on small quantities of normal blood and normal tissues; that is, the filtrates obtained after the material has received such treatment give no reaction on the addition of the alkaline orcin. At times a slight yellow tint makes its appearance, but it cannot be confused with the bright red of the arsphenamine reaction. The above-mentioned amounts of acid and nitrite are quite sufficient promptly to diazotize arsphenamine which has been added to blood or portions of finely ground organs.

Of the protein precipitants, trichloroacetic acid has given the most reliable results, but it has been found necessary to add methyl alcohol with this reagent, since with trichloroacetic acid alone, a certain amount, usually about 20 per cent, of the arsphenamine is carried down with the precipitate. The methyl alcohol prevents materially the occlusion of the drug by the precipitate. If the nitrite solution has been added in excess, the alcohol is distinctly disadvantageous, for the final color will not be the usual red but orange. To remedy this, mild oxidizing agents have been tried with the hope that the excess nitrite would be converted to nitrate. This procedure has met with indifferent success. The preferable method is to control accurately the amount of sodium nitrite in the initial manipulation.

METHOD APPLIED TO WHOLE BLOOD

The following procedure has been found to yield trustworthy results with arsphenamine-containing blood specimens obtained either by the addition of the drug *in*

vitro or by drawing blood from animals or from man after intravenous injection. One cubic centimetre of oxalated blood is placed in a 10 cc. volumetric flask or calibrated test-tube. Approximately 3 cc. of distilled water are added to luke the blood. Next, 0.5 cc. each of one-tenth normal hydrochloric acid and one per cent sodium nitrite are mixed with the laked blood. Cooling the mixture before the nitrite solution is added has always been recommended and is, without doubt, a matter of importance when concentrated reagents are employed. If the solutions are not actually chilled with ice, care should be exercised that they do not become warm at any time. The nitrite solution should have been freshly prepared, since the salt decomposes in aqueous solution. If the reagents are of sufficient strength a faint odor of nitrous acid can be detected coming from the mixture. The next step consists in the addition of 1 cc. of trichloroacetic acid, 20 per cent, and 1 or 2 cc. of methyl alcohol. The volume is now made up to 10 cc. and the contents of the flask are thoroughly mixed. After a few minutes they are poured on a small filter.

Of the clear filtrate 5 cc. are measured into another flask or calibrated tube. Filtrates containing even a trace of hemoglobin must be discarded because of the error it will introduce. To the filtrate are added 2 cc. of one-fourth normal sodium hydroxide and 0.5 cc. of 0.05 per cent orcin. These last named reagents should always be used in the order mentioned, for the orcin, if added when the solution is still acid, will be attacked by the nitrous acid present, the result being a yellow color. If, however, the procedure has been carried out properly and arsphenamine was present in the blood, the red color will promptly develop. The tube can be filled to a definite volume in 10 minutes and compared with suitable standards made up, preferably, in normal blood and treated at the same time.

METHOD APPLIED TO TISSUES

Five-gram samples of the various organs are cut up with scissors and placed in a small mortar. To this is added an amount of washed sand which has previously been found to displace 1 cc. of water. The material is now ground with a pestle until it is reduced to a pulp. A few cubic centimeters of distilled water are used to facilitate the transfer of the material to an accurate 25 cc. measuring cylinder. The mortar and pestle are both washed several times with small amounts of distilled water, care being exercised to keep the volume below 15 cc. To the contents of the cylinder are added 1 cc. of 10 per cent hydrochloric acid and 1 cc. of 2 per cent sodium nitrite. The mixture is shaken, and after 2 or 3 minutes the addition of 3 cc. of 20 per cent trichloroacetic acid and 2 cc. of methyl alcohol is made. The solution is made up to 25 cc. and poured on the filter after standing but a few minutes.

A 12 cc. aliquot of the filtrate represents one-half of the tissue sample, since 1 cc. was made up by the sand. This amount of filtrate is placed in a measuring cylinder and mixed with sufficient one-fourth normal sodium hydroxide to alkalinize. Then 1 cc. of 0.05 per cent orcin is added and the volume made up to 20 cc. The amount of alkali for all organs and for the various conditions under which they may be obtained cannot be stated definitely. It is advisable, therefore, to determine the amount on the unused portion of the filtrate. In making comparisons it is also advisable to control the standards made in aqueous solution with standards made with finely divided normal tissues, since the procedure is both a check on the activity of the reagents and on the yield after the precipitation of the proteins.

SPINAL FLUID AND BLOOD PLASMA

Both of these fluids can be treated without the use of a protein precipitant when they are totally free from hemoglobin or red blood cells. The cloudiness resulting from the addition of the hydrochloric acid is dissipated by the subsequent alkalization. Large quantities of these fluids can be used and the final dilutions can be made as small as is compatible with the determination. Thus, the delicacy of the method is greatly enhanced, a most desirable circumstance in dealing with the spinal fluid which rarely contains more than a trace of arsphenamine.

URINE

Small quantities of urine give more accurate results than large ones. This is due to the fact that a large quantity of urine introduces a considerable amount of yellow color, which, despite the fact that the standard may have been made up with an equal quantity of normal urine, makes accurate approximation exceedingly difficult.

Since the completion of this work Autenrieth and Taege⁴ have published a quantitative method for the estimation of arsphenamine in urine which is also based on the Abelin reaction. These workers report that the red color they obtain is not permanent. They, however, use the reagents in considerable excess, and, for this reason, find that the solutions undergo rapid alteration.

DISCUSSION

Although the diazo reaction employed by us is not given by substances normally occurring in the body fluids, at least under the conditions in which we employ the test, and is readily given by arsphenamine, neoarsphenamine, and the silver sodium preparation, it is also of importance to know what decomposition products of these drugs also react. During the course of the work it was noted that old solutions, which in certain instances had been made several weeks before, gave reactions with very little less intensity than fresh arsphenamine. It is well known that such solutions undergo change with the formation of less complex radicles. By

actual determination it was shown that oxy-amino-phenyl-arsenoxide gave the test as readily as the mother substance. This compound has long been reputed to be the first oxidation product.

Table 2 is a summary of the results obtained in an animal experiment in which the blood, cerebrospinal fluid, urine, and the various organs were analyzed by the method just described. The animal received disodium arsphenamine in a dosage of 20 mgm. per kilo. The results are presented as illustrative of the application of this method to experimental work. The concentration and distribution of arsphenamine will be discussed in a subsequent communication.

TABLE 1

Arsphenamine used	1 mgm.	0.5 mgm.	0.1 mgm.	0.05 mgm.
Final dilution	500 cc.	250 cc.	50 cc.	25 cc.
Colorimetric reading against the 1 mgm. sample as standard —at 40 mm.	40 mm.	39.4 mm.	40.2 mm.	40.5 mm.
Arsphenamine found —Milligrams	1.0	0.508	0.0995	0.0494

TABLE 2

Dog, male, weight 11.1 kilos—	
Time Disodium arsphenamine 20 mgm. per kilo.	
1:50.....	normal blood sample taken.
1:55.....	injection of arsphenamine begun.
2:02.....	injection complete.
5:02.....	cisterna puncture, spinal fluid obtained.
5:05.....	killed with chloroform, organs removed.
Arsphenamine in the blood.	
Time after injection was completed.	Milligrams per 100 c.c.
10 minutes	5.6
33 "	4.5
60 "	4.0
120 "	3.5
180 "	3.1
Arsphenamine in the tissues.	
Organ	Milligrams per 100 gm.
Spleen	6.0
Liver	4.0
Lung	3.2
Heart	3.1
Kidney	3.0
Pancreas	2.9
Intestine	2.6
Muscle	2.5
Brain	less than 1.0
Spinal fluid	negative.
Bladder urine	6.0 mgm. per 100 cc.

REFERENCES

1. Myers, C. N.: 1919. Determination and Distribution of Arsenic in Certain Body Fluids After the Injection of Arsenobenzol, Salvarsan and Neosalvarsan. Pub. Health Reports, XXXIV, 881.

2. Clausen, S. W. and Jeans, P. C.: 1922. The Distribution and Excretion of Arsenic After Intravenous Administration of Arsphenamine in Children. Amer. Jour. Syph., VI, 556.

3. Abelin, J.: 1911. Ueber eine neue Methode das Salvarsan nachzuweisen. Muench. med. Wehnschr., LVIII, 1002.

4. Autenrieth, W. and Taege, H.: 1922. Ueber Ausscheidung und Bestimmung des Salvarsans im Harn. Muench. med. Wehnschr., LXIX, 1479.

A CLINICAL STUDY OF ECTOPIC PREGNANCY

By LEO BRADY, M.D.

(From the Department of Gynecology of the Johns Hopkins Hospital and University)

Since 1883 when Lawson Tait was the first to operate on a case of ruptured tubal pregnancy, so much advance has been made in the study of the etiology, pathology, diagnosis and treatment of ectopic gestation that there seems to be very little left for future investigators to work out along these lines. Now that our knowledge of this subject has reached its present height it may, however, be of interest to study a series of patients operated on during recent years and find out in how far the knowledge gained has been helpful to the clinician and surgeon in the way of diagnosis and therapy.

With this end in view we have studied carefully the cases of ectopic gestation which have come to operation during the last five years at the Johns Hopkins Hospital in the gynecological clinic, with the object of determining in what percentage the text-book symptoms and signs have been found, and of judging the results of operative interference. During the years 1917 to 1922 there were fifty operations performed. This work was done by thirteen different surgeons, the five senior members of the staff and the eight men who during those years in turn held the position of resident gynecologist.

The average age of the women in this series was 26 years. The two oldest were 39 and 38, the two youngest 17 and 19. There were 33 white, and 17 colored women. As only one colored patient is admitted to the hospital to three white, it is evident that ectopic gestation occurred somewhat more frequently among the negroes.

Two women only had been operated on previously. It is of interest that in both patients the previous operation had also been for ectopic pregnancy. In one instance there had been an elapse of thirteen years between the two pregnancies, in the other of only two. Many observers have noted that women who have had an ectopic pregnancy on one side not infrequently in later years have one on the other side.

In six patients only out of the fifty did the ectopic pregnancy represent their first conception. This is in accordance with what is usually taught. The average number of normal pregnancies before the occurrence of the ectopic gestation in our series was two or three, although one woman had had eleven and another ten normal children before being admitted to the hospital with a ruptured tubal pregnancy. The average length of time in our series since the last normal pregnancy was three and a half years. In two women no conception had occurred for ten years and in two others ectopic gestations had taken place while they were nursing babies only three months old.

In only seven (14 per cent) of our cases do we feel certain that there had been a previous pelvic infection, although it is rather difficult to judge of this fact from records, especially those taken on patients critically ill.

In 24 out of the 50 cases there was a history of missed menstrual periods, usually one or two. Three was the largest number missed. Irregular menstrual bleeding had been present in 37 women, absent in 13. The duration of the metrorrhagia varied from a few days to five months, the average being one month.

There was a history of pain in all the cases except two. In 38 the pain was severe, in ten slight. Fainting occurred only three times, twice in cases of tubal rupture, once in a case of tubal abortion. Vomiting is recorded nine times, that is, in 18% of the cases, a higher percentage than is believed to be usual.

The average temperature was 99.4° F. Nineteen patients, or over one in three, had temperatures above 99°, six had 101° and above. There were no instances of a temperature below 98°. The average pulse rate was 104. Sixty-six per cent of the patients had pulse rates above 90°. On admission the pulse rate of one woman was counted 150, that of two others 140, that of six others 120. A striking change in respiratory rate occurred only once. In this instance a woman in profound shock on admission was breathing only six times to the minute.

The leucocyte count averaged 11,000. In sixteen instances the count was above 10,000. The highest counts recorded are 25,000, 24,000 and 20,000. The average hemoglobin content was 61%. In fourteen the figure was below 50, in seven below 40, in three below 30 (28, 27 and 24%). In nine instances the blood pressure was below 110. The low readings were 85 systolic over 50 diastolic and 75 systolic over 55 diastolic.

The general condition was noted as good in 15 cases, fair in 13, poor in 5, and in 3 critical. In the other histories there was no record found on this point, so presumably the women were in good or at least fair condition. Examination of the breasts showed secretion present in eight instances. Two of these women had never been pregnant before and in their cases this finding was of real diagnostic help. A considerable percentage of women who have borne children continue to have secretion in the breasts for a number of years afterwards, so this finding in the other six women was of less significance. The abdomen in nine instances was distended. Tenderness was present in 42 out of 50 cases, but muscle spasm in only 9. Percussion revealed shifting dullness in the flanks four times. In no instance was bluish discolor-

oration of the umbilicus noted.* Bluish discoloration of the umbilicus as a diagnostic sign where ruptured extra-uterine pregnancy exists was first described in 1918 by Dr. Thomas S. Cullen.¹ Since that time several writers have noted it and found it a great aid in reaching a diagnosis.²

Vaginal bleeding was present at the time of examination in twenty-seven women. The cervix was normal in thirty-eight, soft in eighteen cases. Enlargement of the fundus was noted in only five of the women, a pelvic mass was palpated in forty-four cases, but blood crepitus was made out only five times. Pelvic tenderness was present in all except seven of our women. In thirty it was moderate, in thirteen intense.

After all, the real test of the value of the above signs and symptoms of ectopic pregnancy is found in the percentage of correct diagnoses. In thirty-six out of the fifty women (72%) operated on during five years at the Johns Hopkins Hospital by the gynecological staff the correct diagnosis was made.

The thoroughness of the work performed and not the speed with which it is done affords the real criteria by which surgery should be judged, and yet in many operations for ectopic gestation speed is not merely an asset for the surgeon, it is a necessity. Since the majority of our patients were in good condition when operated on, in not a few cases other conditions besides the ectopic pregnancy were remedied, so that the average recorded operative time would mean nothing. It is perhaps of some interest, however, to know that in twenty-five cases the entire operation lasted forty minutes or less, in twelve half an hour, and that in two cases the time between the making of the incision and tying of the last knot was twenty minutes.

At operation the pregnancy was found to have been on the right side, in 27; and on the left, in 23 other instances. There were nine instances of unruptured tubal pregnancy and twenty each of tubal rupture and tubal abortion. In most of the series reported tubal abortion has been found to occur more frequently than tubal rupture. The fiftieth case, which is missing from the above figures, is one of special interest and one which we shall mention in some detail.

Gyn. 26907—The patient, aged 25, was admitted May 31, 1921, complaining of pain in the right side and back. She had been married eight years and had had one child, at that time two years old. When she entered the hospital she had missed one

menstrual period. There was no history of irregular bleeding. For three weeks there had been pain in the right lower quadrant of the abdomen. With the patient under ether Dr. Kelly found that the fundus of the uterus blended with a large mass which occupied the right half of the pelvis and was the size of a three months' pregnancy. A diagnosis of extra-uterine pregnancy was made. At operation he found a pregnancy, 12 by 7 cm., occupying the cornu and right half of the uterus. The left half of the uterus was not enlarged. Both tubes and ovaries were normal. Dr. Kelly diagnosed the condition as an apical pregnancy and deliberated considerably before he determined what he should do. He stated that he had seen only two such cases before and that both these patients had gone to term without any interference and been delivered by normal procedures. He then simply closed the abdomen. The other day the patient showed me the baby whose delivery, she stated, had been accompanied with no difficulty.

The tube and ovary were removed together in thirty, the tube only in nineteen cases. In the case of apical pregnancy, as has been said, the only operation was an exploratory laparotomy. In several cases hysterectomies and appendectomies were done because of conditions found at operation associated with the ectopic gestation. The operative procedure of Dr. Cullen³ in one case is of special interest and has already been fully reported by Dr. H. N. Shaw.⁴

Before admission to the hospital the patient had had a previous operation at which time the right tube and ovary had been removed. On opening the abdomen a cornual pregnancy was found on the left side, and Dr. Cullen resected the left cornu. Knowing that the patient was very desirous of having children he implanted the distal half of the left Fallopian tube into the uterus. Eight months after the operation the patient became pregnant but miscarried at the seventh month, on account of a placenta praevia. Fourteen months after this miscarriage she gave birth to a full term child.

The convalescence in 40 cases was uneventful, in 10 stormy. Abdominal distention occurred six times, prolonged vomiting five times. One of the fifty incisions became infected, but the infection cleared up in a short time. Bronchopneumonia occurred twice. One patient had a post-operative hemorrhage on the second day after operation from the uterine cornu which necessitated surgical interference to control the bleeding.

Twelve infusions and seven transfusions were given. As a rule, the infusions were not started before the operation because it was thought that raising the blood pressure would promote further bleeding into the abdominal cavity. Stimulating drugs were used after the operation on twelve patients. One woman on admission was in such a serious condition that a transfusion was necessary before surgical interference could be carried out. In the other instances the transfusion was given after the operation. In only one instance did a severe reaction occur after the transfusion, although most of the women had a chill and a moderate rise in temperature.

We have divided our cases into three groups, those of tubal abortion, those of tubal rupture and those of unruptured tubal pregnancy, and have made a compara-

* Jackson has suggested placing the patient in a dark room and directing a strong light toward the umbilicus as a means of demonstrating Cullen's sign in a larger percentage of cases. Undoubtedly this suggestion will frequently be of help. As some writers have pointed out, this sign is really not specifically one of ruptured ectopic pregnancy, but merely of intra-abdominal hemorrhage. Nevertheless, in the large majority of instances abdominal hemorrhage in young women is due to ruptured tubal pregnancy.

tive study of the occurrence of the symptoms and signs of ectopic pregnancy in these groups. The rare case of apical pregnancy is not included.

There was no difference in the number of periods missed before operation, or in the duration of irregular bleeding in the three classes, and pain was equally constant throughout. In the women with unruptured ectopic pregnancy, fainting did not occur, nor do we find in the record of that group any instances of temperature elevations or increased pulse rate. Although it is usually stated that the cases showing greater shock are those of tubal rupture rather than those of tubal abortion, a very rapid pulse rate with a low blood pressure was noted on admission with about equal frequency in the two groups. The higher leucocyte counts are all found in the two classes of ruptured tubal pregnancy and are of equal frequency in tubal abortions and tubal ruptures.

The following table gives the comparative hemoglobin findings in the three groups:

	Tubal abortion 20 cases	Tubal rupture 20 cases	Unruptured tubal pregnancy * 10 cases
Average haemoglobin reading.....	56%	58%	70%
No. of patients with haemoglobin below 50	6	7	0
No. of patients with haemoglobin below 40	4	2	0
No. of patients with haemoglobin below 30	3 (28%, 25%, 24%)	1 (27%)	0

*The case of apical pregnancy is here included.

As has been said, all of our patients except two (both women with unruptured tubal pregnancies) showed abdominal tenderness, but distention, dullness in the flanks and muscle spasm were present in only a small percentage and never when the pregnancy was unruptured.

Only when blood crepitus was felt did the pelvic exam-

ination help much in determining whether the pregnancy had or had not ruptured. The abdominal examination, general condition of the patient and history were of much greater aid in making this decision.

Our diagnosis of extra-uterine pregnancy was correct in 72 per cent of the fifty cases taken altogether. In the tubal abortions and rupture cases the figure was 75, in the unruptured 60 per cent.

All of these patients operated upon for extra-uterine pregnancy during the last five years made a complete recovery. On going back to our earlier records we found that in February 1913 a patient was operated on for a ruptured extra-uterine pregnancy. The sac contained a badly macerated fetus and 100 cc. of very foul pus. Although abdominal and pelvic drainages were both used, the patient succumbed to general peritonitis. Between February 1913 and September 1922, there have been seventy-one consecutive successful operations.

In conclusion I wish to extend my thanks to Dr. Thomas S. Cullen, the chief of the gynecological department, for the interest he has taken in the preparation of this report.

BIBLIOGRAPHY

1. Cullen, Thomas S.: Bluish Discoloration of the umbilicus as a Diagnostic Sign where Ruptured Extra-uterine Pregnancy exists. Contribution to Medical and Biological Research Dedicated to Sir William Osler, in Honor of his Seventieth Birthday.
2. Novak, Emil: Bluish Discoloration of the Umbilicus in the Diagnosis of Ruptured Extra-uterine Pregnancy. Journal American Medical Association, 1922, 78—March 22nd.
3. Cullen, Thomas S.: A normal Pregnancy Following Insertion of the Outer Half of a Fallopian Tube into the Uterine Cornu, Johns Hopkins Hospital Bull., 1922, XXXIII, September.
4. Shaw, Henry N.: Pregnancy Following Implantation of the Outer End of the Only Remaining Fallopian Tube into the uterine Cornu after Resection of a Cornual Pregnancy, Johns Hopkins Hospital Bull., 1921. XXXII, September.

CONCERNING THE ANTISEPTIC ACTION OF SOME BENZYL COMPOUNDS

By DAVID I. MACHT and JUSTINA H. HILL

(From the Pharmacological Laboratory and The Brady Urological Institute, Johns Hopkins University)

INTRODUCTION

Nearly five years ago Macht first announced his discovery of the local anesthetic properties of benzyl alcohol.¹ Not long after he published his studies concerning the antispasmodic properties of some benzyl esters² and a little later described the pharmacological effects of benzaldehyde, which was proved to exhibit both the local anesthetic and antiseptic properties shown by benzyl alcohol and benzyl esters respectively.³ Soon after the publication of the study on benzyl alcohol as a local anesthetic Macht and Nelson pointed out an additional interesting property possessed by this drug, namely, its antiseptic action.⁴

This property of benzyl alcohol was further studied and corroborated by Macht, Schwartz and Satani in connection with a comparative study of the antiseptic properties of some local anesthetics.⁵ In view of the fact that since the publication of the above papers various benzyl compounds have come to be extensively used therapeutically, it was deemed desirable to inquire whether the various esters as well as benzaldehyde are also antiseptic or germicidal as benzyl alcohol had been found to be. Accordingly, the present research was undertaken.

In this investigation a bacteriological study of the following substances was made: benzyl alcohol, benzalde-

TABLE I.

DRUG	Dilution	1 Minute Exposure		1 Hour Exposure		3 Hours' Exposure		5 Hours' Exposure	
		Number of organisms per c.c.	% of organisms	Number of organisms per c.c.	% of organisms	Number of organisms per c.c.	% of organisms	Number of organisms per c.c.	% of organisms
Benzaldehyde	1:10	0	0	0	0	0	0	0	0
Benzaldehyde	1:10	0	0	0	0	0	0	0	0
Benzaldehyde	1:100	368,145	100	250	0.067	0	0	0	0
Benzyl acetate	1:10	336,330	100	0	0	0	0	0	0
	1:100	1,181,815	100	63,630	5.392	150	0.012	0	0
Benzyl alcohol	1:10	0	0	0	0	0	0	0	0
Benzyl alcohol	1:10	0	0	0	0	0	0	0	0
Benzyl alcohol	1:100	409,050	100	268,155	65.555	14,850	3.630	0	0
Benzyl benzoate	Not diluted	527,220	100	18,180	3.448	0	0	0	0
Benzyl benzoate	1:2	427,230	100	96,355	22.553	18,270	4.276	0	0
Benzyl benzoate	1:10	449,955	100	—	—	—	—	185	—
Tincture of Benzoin	1:2	0	0	0	0	0	0	0	0
Tincture of Benzoin	1:2	0	0	0	0	0	0	0	0
Tincture of Benzoin	1:10	2,272	100	0	0	0	0	0	0
Tincture of Benzoin	1:100	390,870	100	0	0	0	0	0	0
Balsam of Peru	Not diluted	0	0	0	0	0	0	0	0
Balsam of Peru	Not diluted	0	0	0	0	0	0	0	0
Balsam of Peru	1:10	145,440	—	31,815	21.875	0	0	0	0
Balsam of Peru	1:100	527,220	—	128,625	24.396	44,540	8.637	80	0.015
Oil of Cajuput	1:2	73,629	100	0	0	0	0	0	0
Oil of Cajuput	1:10	1,408,950	100	22,725	1.612	500	0.035	0	—
Oil of Cajuput	1:100	427,230	100	—	—	—	—	10,000	2.340
0.875 % NaCl control		2,363,635	100	4,363,630	184.615	1,363,635	57.694	127,260	5.384
Cotton-seed Oil control		136,350	100	145,440	100.666	30,910	22.669	25,450	18.665
10% Ethyl alcohol control		736,290	100	49,050	6.661	150	0.002	0	0

hyde, benzyl benzoate, benzyl acetate and a number of natural pharmacological compounds containing benzyl bodies such as benzoin, balsam of Peru, balsam of tolu and a number of others.

METHOD OF STUDY

In the work of Macht, Schwartz and Satani, three different methods for testing the antiseptic action were employed, the most important of which was the introduction of bacterial cultures into solutions of the drugs in different concentrations and the replanting of the same organisms after centrifugalization and washing on agar media. In the present work a different method was employed, inasmuch as none of the substances studied were very soluble in water but were freely soluble in oils. Dilutions of the drugs were made with sterile cottonseed oil. The procedure employed was briefly as follows: Two cubic centimeters of the test dilution of the drug to be examined were placed in a test-tube and inoculated with one loopful of a 24-hour broth culture of staphylococcus pyogenes aureus, which had been filtered through glass wool to remove clumps of organisms. After careful shaking for one minute, two agar plates were made, each containing 0.1 c.c. of the drug-organisms mixture removed by means of a capillary pipette attached to a tuberculin syringe. In addition two parallel series of dilutions were made with sterile salt solution and from these dilutions

agar plates were also made. The tests were done at room temperature, averaging 30° C. After 48 hours' incubation at 37.5° C. the most suitable plates for counting were selected, the average number of colonies from the plates of the same dilution in the parallel series was determined, and the corresponding number of bacteria per cubic centimetre was calculated.

The results obtained are summarized in Table I. The table gives the results obtained with benzaldehyde, benzyl acetate, benzyl alcohol, benzyl benzoate, tincture of benzoin, balsam of Peru and oil of cajuput. The oil of cajuput was selected as representative of a number of volatile oils occurring in nature which have been found to contain benzyl derivatives. As check controls for the tincture of benzoin, experiments were also made with 10 per cent ethyl alcohol. Other controls were made with cottonseed oil and with normal saline solution. In this table are indicated the concentration or dilution of the drugs used, the number of organisms surviving after several periods of exposure to the drugs, and also the percentage of such organisms after comparison with the original cultures.

RESULTS

From an examination of the table it will be noted that benzyl alcohol even when diluted with oil exhibits definite antiseptic properties, thus confirming the results previously obtained. A dilution of benzyl alcohol 1:10 was

sufficient to kill the organisms after exposure of one minute. Dilutions with oil 1:100 gave no growth after exposure of the bacteria for five hours.

Benzaldehyde in dilution of 1:10 was also germicidal on exposure for one minute and in dilution of 1:100 proved to be even more effective than that of benzyl alcohol, inasmuch as after one hour's exposure only a small number of colonies developed and after three hours there was complete inhibition of growth.

Even benzyl benzoate and benzyl acetate exhibited some antiseptic effects, as may be seen from the table. Benzyl acetate diluted (1:10) gave no growth after one hour's exposure and a markedly reduced number of organisms even with a dilution of 1:100. Benzyl benzoate undiluted killed all of the organisms after three hours' exposure and showed slight germicidal properties in dilutions of from 1:10.

The galenical preparations of benzoin and also balsam of Peru likewise exhibited some antiseptic effect. Tincture of benzoin even in dilution of 1:100 inhibited growth of organisms after an exposure of one hour. Similar properties, though to a lesser degree, were shown by oil of cajuput.

COMMENT

The obvious source of error in the above experiments is the fact that drug-organism mixtures were for the most part emulsions so that the evenness of the mixture was subject to some variation in spite of careful mixing. For this reason the results obtained should be considered only as indications of the relative value of the drugs in question under nearly the same conditions as was experimentally possible. Nevertheless, they are striking and certainly speak for a definite germicidal and antiseptic effect in the case of every one of the compounds examined. This peculiar property of the various benzyl compounds is not only of scientific interest but also of practical value. Thus, for instance, tincture of benzoin has been empirically employed in the form of inhalations for the treatment of sore throat. The beneficial results following such a practice are not to be ascribed altogether to the effects of the moist heat applied in the form of steam, but may very well be partly explained by the antiseptic properties

of benzaldehyde. In addition to this, as has been shown by Macht elsewhere,⁶ the local anesthetic effects of benzaldehyde probably also play a rôle in this connection. Such an employment of benzoin could be improved upon by the fortification of the benzoin tincture with small amounts of benzaldehyde, or still better of benzyl alcohol. Again, tincture of benzoin has been long employed empirically as an ingredient of various lotions and skin preparations. Such preparations aside from being agreeably perfumed by the drug are probably beneficial therapeutically by virtue of the antiseptic properties of benzaldehyde described above and also by virtue of the local anesthetic properties of benzaldehyde, described by one of the authors elsewhere.

In view of the above, the antiseptic properties of balsam of Peru probably offer a rational explanation of the efficient results obtained with this drug in dermatological practise as a parasiticide in such conditions as scabies, and others.

One of the authors has already called attention to the sedative action of benzyl benzoate and other benzyl esters in certain intestinal conditions such as diarrheas and dysenteries. It has been noted by Haughtwout, Lantin and Asuzano in Manila that benzyl benzoate was quite beneficial in the treatment of both amœbic and bacillary dysentery.⁷ This beneficial action may be due not only to the antispasmodic properties of the drug but also to its antiseptic action. Perhaps the presence of benzyl alcohol in oil of cajuput may explain in the same way the use of that oil in diarrheas and other intestinal conditions.

Inasmuch as tincture of benzoin is extensively employed in laryngological practise for inhalation, etc., it was interesting to compare its efficiency as an antiseptic with that of some other drugs employed for this purpose by the clinician. A bacteriological examination was therefore made of solutions of guaicol and menthol in oil. These drugs are among the most commonly employed by laryngologists. The results obtained are shown in Table 2. It will be noted that neither guaicol nor menthol solutions in albolene alone nor in mixtures of the two together in cottonseed oil exhibited any such germicidal and antiseptic effects as benzyl alcohol, tincture of benzoin or benzaldehyde did. The latter would therefore be expected to be more efficient as therapeutic agents in this respect.

TABLE II.

DRUG	Dilution	1 Minute Exposure		1 Hour Exposure		3 Hours' Exposure		5 Hours' Exposure	
		Number of organisms per c.c.	% of organisms	Number of organisms per c.c.	% of organisms	Number of organisms per c.c.	% of organisms	Number of organisms per c.c.	% of organisms
Guaiacolin albolene	1:100	157,000	100	44,000	28.025	27,000	17.197	2,050	1.305
Mentholin albolene	1:100	38,500	100	5,000	12.987	38,500	100	76,000	197.402
Menthol and Guaiacolin cotton-seed alcohol	1:100 Both	3,000,000	100	2,150,000	71.666	250,000	8.333	16,000	0.533
Albolene control		59,000	100	15,000	25.423	67,500	114.406	67,500	114.406
Cotton-seed Oil control		136,350	100	145,440	106.666	30,910	22.669	25,450	18.665

CONCLUSIONS

1. A bacteriological study of the effect of various benzyl compounds was made on *Staphylococcus aureus*.
2. It was found that all of these exhibit more or less a germicidal and antiseptic effect; benzyl alcohol and benzaldehyde being the most potent in this respect.
3. This property of the benzyl compounds throws light on certain successful empirical usages of a number of drugs.
4. The antiseptic power of benzaldehyde and benzyl

alcohol is much more powerful than that of some other drugs employed as throat antiseptics.

LITERATURE

1. Macht: Jour. Pharm. & Exper. Therap., 1918, XI, 263.
2. Macht: *ibid.*, 1918, XI, 389, 419.
3. Macht: Proc. Soc. Exper. Biol., 1919, XVI, 85.
4. Macht and Nelson: *ibid.*, 1918, XVI, 25.
5. Macht, Schwartz and Satani: J. of Urology, 1920, IV, 347.
6. Macht: Archives internationales de pharm. et de therap., 1922.
7. Haughwout, Lantin and Asuzano: Arch. Int. Med., 1919, XXIV, 383.

THE BLOOD PICTURE OF UNCOMPLICATED PELLAGRA WITH A REVIEW OF THE LITERATURE

By JOHN G. HUCK, M.D.

(From the Biological Division of the Medical Clinic
of the Johns Hopkins University and Hospital)

The rather extensive literature on the blood picture in pellagra is characterized by a lack of agreement on several points.

First, opinion varies as to the grade of anæmia. Carletti,¹ Wood,² Hillman,³ Young,⁴ G. Fratini,⁵ and Grigorescu, and P. Galesescu⁶ record a mild grade of secondary anæmia. Sepilli,⁷ Agostini,⁸ D'Ancona and Randi,⁹ Lombroso,¹⁰ Galesesco and Slatineano,¹¹ Lavinder,¹² Castellani and Chalmers,¹³ E. Mai,¹⁴ Hyde,¹⁵ Roberts,¹⁶ Young, G. Fratini, M. Fiorini, and G. Gavini,¹⁷ and Manson¹⁸ found a moderate grade of secondary anæmia. A more marked grade of secondary anæmia has been observed by Bardin,¹⁹ Babes and Sion,²⁰ and Fiorini and Gavani. Normal hæmoglobin and erythrocytic counts were noted by Hillman, Young, Fratini and Daspit.²¹ Lavinder, Roberts and Lavinder, and Babcock²² refer to the anæmia of pellagra as suggestive of the chlorotic type. E. Mai, Fiorini, and Gavini and Giannini²³ state that in severe cases the anæmia is marked.

Opinions vary also as to the white blood cells in this disease. Carletti, Sepilli, Bardin, Fiorini and Gavini, Daniels and Newham²⁴ found no change in the leucocyte count. Daspit and E. Mai find a decrease in the white count, but E. Mai notes a decrease only in severe cases.

Then other investigators, such as Fratini, Lavinder, Wood, Hillman, Lavinder and Babcock, Young, and G. Fratini state that the white blood cells may be either normal or increased in number, and according to Giannini "the white count shows a leucocytosis in all cases ranging from 10,000 to 24,000 per cubic millimeter." The investigators can also be put into three groups according to their findings from the differential counts. In the first are grouped those that found an increase in the eosinophil cells, such as Fratini, Masini,²⁵ Carletti, and Fiorini and Gavini. In the second group are those in-

vestigators who find an increase in the L. Mono. and Trans. cells, such as Carletti, Galesescu and Slatineano, Sambon,²⁶ Grigorescu and Galesescu, Lavinder and Babcock, Castellani and Chalmers, and E. Mai. In the third group are those who found an increase in the lymphoid cells, such as L. Peserico,²⁷ Wood, Daniels and Newham, Hillman, Fiorini and Gavini, Bardin, Daspit, Roberts, Low,²⁸ Hyde, Young, and Giannini.

M. V. Masini, who studied principally the eosinophilic cells in the blood, draws the following conclusions from the study of 77 cases: "There is a decided eosinophilia which is contrary to what is found in many infectious diseases. This eosinophilia occurs in cycles corresponding with the increase or diminution of the pellagra-genous toxines,—that is, 'the more pronounced the toxemia, the greater the eosinophilia.'" He suggests that this constant eosinophilia may prove at times a valuable aid in early or differential diagnosis.

G. Fratini studied the blood in 34 cases of both acute and chronic stages of pellagra, and states that the "leucocytic formula was always found to be changed quantitatively; especially frequent was eosinophilia; *i.e.*, the presence of large cells laden with granules staining a bright red with orange, and with fuchsin, granules which issue from the cells when the fresh blood is pressed between two glasses before staining. It is known that such cells are indicative of inflammatory imitative alterations of the epithelia investing the mucosæ, and also the glandular epithelia of the intestines, so that they are frequent in helminthiasis, in cases of echinococcosis of the liver, in bronchial asthma, gonorrhœa, ankylostomiasis, infection by *Taenia solium*, and *Bothriocephalus*. It is probable, therefore, that the eosinophilia of pellagrins, as high as 6%, may be related with the alterations of the intestinal epithelium, so frequent and characteristic in pellagra victims."

In a study of 26 cases of pellagra without any intestinal infection or blistering erythema M. Fiorini and G. Gavini observed: (1) The pellagrigenic virus in the majority of cases changes the hæmoglobin content of the blood, reducing it by as much as one-third of the normal value. (2) With equal frequency it alters also the morphological constitution of the blood, producing a marked diminution of the erythrocytes. (3) In exceptional cases it modifies to a marked degree the diameter of the red corpuscles and introduces into the circulation erythrocytes of embryonic form, while no elements tending to show a perversion of the cytogenic function of the bone marrow are noted. (4) The pellagrigenic virus does not produce leukocytosis nor does it markedly alter the corpuscular ratio; (5) On the other hand, it modifies the digestive leukocytes, almost completely abolishing them. (6) It produces a typical mononucleosis (large and small lymphocytes). (7) It produces a decided eosinophilia.

L. Pèserico studied the blood in 13 patients in an asylum. They were old, or on the threshold of old age. He comes to the following conclusion as regards the morphology of the blood:—“(1) The neutrophils varied between 53.7% and 67.4%, and consisted mostly of cells with 2, 3 or 4 nuclei. (2) The number of lymphocytes was always greater than normal. (3) Concerning the large mononuclear non-granular leucocytes, and the transition forms, (the *bête noire* of hematology, as says Pappenheim) I shall make no comments. (4) Finally, I must say that not infrequently, in fact in from 15% to 24% of the cases, I have found that several neutrophils in the same preparation, often in the same microscopic field, showed the protoplasm stained differently from that of the others. In these cells the color approached the eosinophil color, but there were not the true granulations of the eosinophils, and the color did not attain the intensity of shade which is observed in these leucocytes.” Then the author goes on to ask these questions: “Are all these observed facts constant? What value have they, or can they have any value? Has the constitution of individuals a direct influence on them? Are there equal alterations in the blood at the beginning of the disease? Can they have a diagnostic value?”

Two investigators working together, Galesesca and Slatineano, who examined the blood in 31 cases, reported a constant diminution in the number of red blood cells and from 70% to 90% hemoglobin (von Fleischl); also a slight increase in the number of leucocytes. In the differential formula they found a constant large mononucleosis from which no deductions could be drawn as to the etiology.

Lavinder, in his article, gives a review of the literature and reports the blood findings in 24 cases. His patients were mostly negro women in the advanced stages with many secondary nervous changes. Many of the patients had been receiving arsenic in some form. This author re-

ports, “a reduction in red blood cells and hemoglobin. The color lost would seem to be proportionately greater than the red cells lost (chlorotic type of anaemia). There was a great variation in the number of leucocytes with an occasional decided leucocytosis not clinically explicable by complications or otherwise. The differential count showed a relative increase in large mononuclears with an absence of eosinophilia, except in cases with such complications as round worms or hookworms.”

Castellani and Chalmers report the blood picture of pellagra as showing “a reduction in the red blood cells with a proportional reduction in hemoglobin. In form the red blood cells are normal, but microcytes, and more rarely megalocytes, may be found; usually there is only a slight leucocytosis in uncomplicated cases, but there is generally a distinct mononucleosis; when the polymorphonuclear cells are increased, it would appear to indicate always a complication.”

Daspi reports the blood findings in 20 cases of pellagra in the insane, as follows: “The blood picture is fairly constant and deviates but little from the normal. There is a slight falling off in the total leucocytes and a relative lymphocytosis, which is more marked in the small mononuclears. The hemoglobin is consistently good and the red blood count shows nothing abnormal. These findings may be said to be comparatively negative and to offer nothing of value from a diagnostic standpoint.”

Bardin analyzed the blood in 14 cases of pellagra. In this investigation he excluded wherever possible patients suffering from tuberculosis, and those having intestinal worms. Moreover, the blood examinations were made as far as possible during the periods when the pellagra symptoms were at their height; that is to say, when there were marked diarrhoea, skin lesions and stomatitis. He reports “a reduction in the number of red blood cells and a diminution in the percentage of hemoglobin with but little change in the leucocytes. There is a variable reduction in the polymorphonuclear cells and a diminution in the polymorphonuclear-eosinophil cells, while the lymphoid elements are increased, both large and small lymphocytes. The transitional cells are reduced.” The author further states that these cases are presented with some reluctance, because they are rather at variance with the results secured by other observers.

Lavinder and Babcock state, from a review of the literature and from their own work: “Secondary anaemia is a frequent, though not a constant condition, in pellagra. Leucocytosis is not often seen and is probably not a phenomenon of the uncomplicated disease; conclusions from differential leucocyte counts at present are uncertain, but there is probably a relatively large mononuclear increase, and no protozoal parasites have yet been found in the blood.”

CHART I.
PELLAGRA AT HEIGHT OF ILLNESS

NAME	Case No.	Date	Sex	Age	Hb. %	R. B. C. millions	C. I.	Nueleat. Reds	Baso-philia	W. B. C. Thous.	P. M. N. %	P. M. E. %	P. M. B. %	L. lym. %	S. lym. %	L. mono. %	Trans. %	Myelo-cytes				Smudges %	Platelets	Stool Examinations	REMARKS
																		Myeloblasts %							
																		N. %	E. %	B. %					
R. B.	1	4/20/21	M.	40	80%	4,576	0.8	None seen	None seen	6,000	57.3	0.33	0	0.66	33.6	3.0	4.33	0	0	0	0.66	Normal	4/20/21 taken every day until 4/24/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	4/24/21	"	"	80%	4,320	0.9	None seen	None seen	6,600	60.3	0.66	0	0	31.6	1.66	5.00	0	0	0	0.66	Normal	Negative for ova or parasites.	Throat smear neg. for V. A.	
A. L. C.	2	5/ 3/21	M.	35	80%	4,160	0.8	None seen	None seen	8,600	54.3	0.33	1.66	1.0	35.3	2.3	5.0	0	0	0	0	Normal	5/3/21 taken every day until 5/7/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	5/ 7/21	"	"	85%	4,480	0.8	None seen	None seen	9,000	50.6	1.00	0.33	0	38.0	4.0	6.0	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
M. E. B.	3	5/10/21	F.	28	85%	5,120	0.8	None seen	None seen	8,000	47.3	0.66	1.00	0.66	41.6	2.33	6.3	0	0	0	0	Normal	5/10/21 exam. every day until 5/14/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	5/14/21	"	"	85%	5,024	0.8	None seen	None seen	8,600	49.3	1.00	0	0	40.3	4.00	5.3	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
R. C. L.	4	6/ 1/21	M.	40	80%	4,576	0.8	None seen	None seen	7,200	59.3	0.33	0	1.0	33.6	0.66	5.0	0	0	0	0	Normal	6/1/21 exam. every day until 6/5/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	6/ 5/21	"	"	83%	4,320	0.9	None seen	None seen	8,000	56.6	1.00	0	0.33	35.6	2.00	4.33	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
H. H. L.	5	6/ 3/21	M.	32	90%	4,640	0.9	None seen	None seen	8,200	51.6	1.00	0.33	0	38.3	2.33	6.33	0	0	0	0	Normal	6/3/21 exam. every day until 6/7/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	6/ 7/21	"	"	90%	4,896	0.9	None seen	None seen	8,600	52.6	0.66	0	1.0	35.6	3.0	7.0	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
R. C. M.	6	4/25/21	F.	30	65%	3,840	0.8	None seen	None seen	8,400	53.3	1.33	0	0.33	36.6	4.33	4.0	0	0	0	0	Normal	4/25/21 exam. every day until 4/29/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	4/29/21	"	"	62%	3,680	0.8	None seen	None seen	8,000	54.0	0.66	0.33	2.33	34.3	2.00	6.33	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
A. C.	7	5/ 7/21	F.	35	80%	4,160	0.9	None seen	None seen	6,600	60.0	0.33	0	0.66	30.3	3.33	5.33	0	0	0	0	Normal	5/7/21 exam. every day until 5/11/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	5/11/21	"	"	82%	4,480	0.9	None seen	None seen	6,000	58.6	2.33	0.33	1.00	32.6	1.00	4.00	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
B. H. P.	8	5/ 9/21	F.	26	75%	4,096	0.9	None seen	None seen	7,200	57.3	0.33	0	1.66	31.6	4.00	5.00	0	0	0	0	Normal	5/9/21 exam. every day until 5/14/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	5/14/21	"	"	77%	4,320	0.8	None seen	None seen	6,800	58.6	0.00	0.33	3.00	33.3	0.66	4.00	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
J. C. M.	9	5/11/21	F.	30	80%	4,256	0.9	None seen	None seen	8,200	59.0	0.33	0	1.00	35.3	2.33	2.00	0	0	0	0	Normal	5/11/21 exam. every day until 5/16/21.	Blood W.R. neg. Gum smear neg. for V. A.	
"	"	5/16/21	"	"	78%	4,064	0.9	None seen	None seen	8,800	59.3	0.00	0.66	0.66	33.6	1.66	4.00	0	0	0	0	Normal	Negative for ova or parasites.	Throat smear neg. for V. A. Urine neg.	
E. M.	10	6/20/21	F.	33	70%	4,256	0.8	None seen	None seen	8,000	60.0	0.66	0.33	1.00	34.6	1.00	2.33	0	0	0	0	Normal	6/20/21 exam. every day until 6/25/21.	Bl. W.R. neg. Gum sm. neg. V.A. Throat sm. neg.	
"	"	6/25/21	"	"	72%	4,480	0.8	None seen	None seen	10,000	57.6	0.00	0.33	2.33	34.0	2.33	3.33	0	0	0	0	Normal	Negative for ova or parasites.	V.A. Pt. had cold on chest on day count was made.	

E. Mai studied the blood picture in 10 cases, following 5 of the cases over a long period of time. He draws the following conclusions: "Examining the results of my findings, it seems to me evident that it is not possible from the percentages of the leucocytic forms to deduce a typical hemoleucocytic formula for pellagra. In pellagra there is often observed an increase of the large mononuclears, and also transition forms. The eosinophilic cells behave in this morbid condition as in other diseases: there is always noted an increase in the period of improvement. Often the increase of the eosinophilic forms found in the pellagrins during the first days after admittance is related to the helminthiasis which is so frequent in these patients. Leucopenia is observed in the severe cases of pellagric typhus, associated with the generation of the leucocytic forms. In these cases the nucleus is often in the degenerative phase and the granulations are scattered. In the severe cases of pellagra one almost always observes alterations in the form and modifications in the color of the red corpuscles. Further hematological studies will certainly afford valuable assistance in illuminating the course of the disease in which certain characteristic modifications of the elements of the blood are evident at every examination in different periods of observation. I have been able to observe no parasitic forms in numerous examinations, and the culture test has always been negative."

In 39 cases Young found "(1) a slight grade of anæmia or none at all. (2) Total leucocytes ranged from 4,700 to 14,100. In pellagra there is a constant marked lymphocytosis; this lymphocytosis persists long after all acute symptoms have subsided, which is not true of that accompanying starvation. The cases in the main have a higher lymphocyte count after the acute symptoms have subsided, than during the very acute stage. This persistent lymphocytosis resembles in its nature that of other chronic infections such as syphilis and tuberculosis, and is probably due to an infection of a chronic nature. In some cases there was a slightly high eosinophilia, but this was variable, and in every case where the eosinophilia was marked we were able to find intestinal parasites. And finally, a high lymphocyte count is usually of good prognostic import."

O. S. Hillman and P. A. Schule have published two reports. In their first there is a series of 49 cases, and in the second, 46 cases. The authors conclude:—"There is a mild degree of secondary anæmia, and this is not characteristic or constant of the disease. Cases of decided anæmia occur for the most part in patients afflicted with some associated condition to which the anæmia was probably referable, rather than to the pellagra *per se*. Inspection of the tables discloses the fact that lymphocytosis is the predominant feature in the majority of the cases. In these cases showing a decided lymphocytosis,

CHART II.
CONVALESCENT PELLAGRA PATIENTS

NAME	Case No.	Date	Sex	Age	Hb. %	R. B. C. millions	C. I.	Nucleat. Reds	Baso- philia	W. B. C. Thous.	P. M. N. %	P. M. E. %	P. M. B. %	L. lym. %	S. lym. %	L. mono. %	Trans. %	Myelo- cytes			Smudges %	Platelets	Stool Examinations	REMARKS
																		N. %	E. %	B. %				
H. R. M.	1	2/28/20	M.	56	65%	4,224	0.77	None seen	None seen	7,600	55.3	10.00	0	1.66	24.3	5.00	3.00	0	0	0	0.66	Normal	2/28/20 each day after until 3/3/20.	Bl. W.R. neg. Spinal fluid W. R. neg. Gum smear neg. for V. A.
"	"	3/ 3/20	"	"	67%	4,288	0.79	None seen	None seen	7,200	56.0	9.33	0	0.66	25.3	4.66	3.66	0	0	0	0.33	Normal	All exam. neg. for ova or parasites.	Thr. sm. neg. Urine neg.
E. B. H.	2	10/13/20	F.	21	65%	3,776	0.87	None seen	None seen	6,400	58.6	8.00	1.00	0	27.3	2.33	2.66	0	0	0	0	Slight incr.	10/13/20 each day after until 10/17/20	Blood W.R. neg. Gum smear neg. for V. A.
"	"	10/19/20	"	"	68%	3,968	0.87	None seen	None seen	6,800	59.6	8.66	0	0	27.6	2.33	1.66	0	0	0	0	Slight incr.	All exam. neg. for ova or parasites.	Throat smear neg. for V. A. Urine neg.
A. V. B.	3	3/18/20	M.	46	70%	4,064	0.87	None seen	None seen	7,000	60.3	6.60	0	0	28.3	2.00	2.60	0	0	0	0	Normal	3/18/20 each day after until 3/22/20.	Urine neg. Gum smear neg. for V. A. Throat smear neg. for V. A.
"	"	3/22/20	"	"	73%	4,224	0.86	None seen	None seen	6,600	58.3	7.30	0	0.66	29.6	1.66	2.33	0	0	0	0	Normal	All exam. neg. for ova or parasites.	Urine neg. Gum smear neg. for V. A.
M. T. C.	4	11/ 1/20	F.	43	70%	4,032	0.87	None seen	None seen	6,000	57.6	5.33	0	0.66	31.3	2.33	2.66	0	0	0	0	Normal	11/1/20 each day after until 11/5/20.	Blood W.R. neg. Gum smear neg. for V. A. Throat smear neg. for V. A. Urine neg.
"	"	11/ 5/20	"	"	72%	4,128	0.87	None seen	None seen	7,000	58.3	7.3	0	0	29.6	3.33	2.55	0	0	0	0	Normal	All exam. neg. for ova or parasites.	Urine neg. Gum smear neg. for V. A. Throat smear neg. for V. A.
H. G.	5	9/ 1/20	F.	26	68%	3,936	0.89	None seen	None seen	7,200	66.3	4.66	0	0.33	24.6	1.66	2.66	0	0	0	0	Normal	9/1/20 each day after until 9/5/20.	Urine neg. Gum smear neg. for V. A. Throat smear neg. for V. A.
"	"	9/ 5/20	"	"	70%	4,064	0.87	None seen	None seen	7,000	65.0	5.66	0	0.66	26.0	2.00	0.66	0	0	0	0	Normal	All exam. neg. for ova or parasites.	Urine neg. Gum smear neg. for V. A.
A. C.	6	10/18/20	F.	23	60%	3,968	0.76	None seen	Slight diffuse	6,400	66.6	4.00	1.00	0	23.6	2.66	1.33	0	0	0	0	Increase	10/18/20 each day after until 10/22/20	Blood W.R. neg. Sputum neg. for tbc., etc. Gum smear neg. for V. A. Throat smear neg.
"	"	10/22/20	"	"	63%	4,032	0.78	None seen	Slight diffuse	6,000	65.3	4.66	0	0.33	25.3	2.33	1.66	0	0	0	0.33	Increase	All exam. neg. for ova or parasites.	Bl. W.R. neg. Spinal fluid W. R. neg. Urine neg. Gum smear neg. for V. A. Throat smear neg.
A. M.	7	8/ 4/20	M.	43	85%	4,416	0.96	None seen	None seen	7,000	67.3	8.33	0	0.66	21.3	1.66	0.66	0	0	0	0	Normal	8/4/20 each day after until 8/8/20.	Bl. W.R. neg. Spinal fluid W. R. neg. Urine neg. Gum smear neg. for V. A. Throat smear neg.
"	"	8/ 8/20	"	"	82%	4,448	0.93	None seen	None seen	6,800	66.6	7.66	0	1.00	22.3	1.33	1.00	0	0	0	0	Normal	All exam. neg. for ova or parasites.	Bl. W.R. neg. Spinal fluid W. R. neg. Urine neg. Gum and Throat smears neg.
M. E. F.	8	7/ 8/20	F.	58	65%	4,032	0.71	None seen	None seen	5,800	63.6	8.66	0	0.33	23.6	1.66	2.00	0	0	0	0	Normal	7/8/20 each day after until 7/12/20.	Bl. W.R. neg. Spinal fluid W. R. neg. Urine neg. Gum and Throat smears neg.
"	"	7/12/20	"	"	62%	4,000	0.77	None seen	None seen	6,000	62.6	8.33	0	0	25.3	1.33	2.33	0	0	0	0	Normal	All exam. neg. for ova or parasites.	Bl. W.R. neg. Spinal fluid W. R. neg. Urine neg. Gum and Throat smears neg.
A. B.	9	1/20/21	F.	27	75%	4,128	0.91	None seen	None seen	6,000	51.3	8.33	0.33	0	29.3	6.33	2.33	0	0	0	2.00	Slight incr.	1/20/21 each day after until 1/24/21.	Bl. W.R. neg. Spinal fluid W. R. neg. Urine neg.
"	"	1/24/20	"	"	72%	4,032	0.90	None seen	None seen	6,200	52.3	7.66	0.66	0.66	28.3	6.66	3.00	0	0	0	0.66	Slight incr.	All exam. neg. for ova or parasites.	Bl. W.R. neg. Spinal fluid W. R. neg. Urine neg.
P. S.	10	1/24/21	F.	31	55%	3,968	0.70	None seen	None seen	7,400	71.3	5.33	0	0.66	15.6	3.66	2.66	0	0	0	0.66	Slight incr.	1/24/21 each day after until 1/28/21.	Bl. W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
"	"	1/28/21	"	"	59%	4,096	0.73	None seen	None seen	7,000	69.6	6.00	0.33	0.33	16.3	4.00	3.00	0	0	0	0.33	Normal	Negative for ova or parasites.	Gum and Thr. smears neg. for V. A. Urine neg.
C. E. H.	11	9/ 3/20	F.	28	76%	4,128	0.92	None seen	None seen	6,000	56.3	4.66	0.66	0	29.3	5.66	3.33	0	0	0	0	Normal	9/3/20 each day after until 9/7/20.	Gum and Thr. smears neg. for V. A. Urine neg.
"	"	9/ 7/20	"	"	80%	4,224	0.95	None seen	None seen	6,400	58.3	5.00	0	0	26.6	6.33	3.66	0	0	0	0	Normal	Negative for ova or parasites.	Gum and Thr. smears neg. for V. A. Urine neg.
J. H. D.	12	6/30/20	F.	31	75%	4,096	0.93	None seen	None seen	6,800	64.3	5.33	0.33	0.66	24.3	3.66	1.33	0	0	0	0	Normal	6/30/20 each day after until 7/4/20.	Gum and Thr. smears neg. for V. A. Urine neg.
"	"	7/ 4/20	"	"	70%	4,000	0.87	None seen	None seen	7,000	62.3	6.33	0	0	26.6	3.00	1.66	0	0	0	0	Normal	Negative for ova or parasites.	Gum and Thr. smears neg. for V. A. Urine neg.
E. E. B.	13	8/16/20	F.	46	70%	4,096	0.87	None seen	None seen	6,000	52.6	4.66	0	1.33	35.3	2.33	3.66	0	0	0	0	Normal	8/16/20 each day after until 8/21/20.	Gum and Thr. smears neg. Urine 1020. Acid, trace albumin. Few fine granular casts.
"	"	8/20/20	"	"	67%	4,032	0.83	None seen	None seen	5,800	51.6	6.00	0	0.66	36.6	1.66	3.33	0	0	0	0	Normal	Negative for ova or parasites.	Bl. and Spinal Fluid W. R. neg. Urine neg. Gum and Throat smears neg. for V. A.
M. T. G.	14	9/ 6/20	F.	37	78%	4,224	0.92	None seen	None seen	7,000	64.3	4.33	0	1.66	24.3	1.00	4.33	0	0	0	0	Normal	9/6/20 each day after until 6/20/20.	Bl. W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
"	"	9/20/20	"	"	75%	4,128	0.91	None seen	None seen	7,200	62.6	5.33	0.33	1.00	28.3	0.66	1.66	0	0	0	0	Normal	Negative for ova or parasites.	Bl. W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
C. N. W.	15	4/27/20	M.	42	64%	4,320	0.74	None seen	None seen	7,400	54.3	7.66	0	0.66	28.6	6.00	2.66	0	0	0	0	Normal	4/27/20 each day after until 5/1/20.	Bl. W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
"	"	5/ 1/20	"	"	65%	4,224	0.77	None seen	None seen	7,600	52.3	8.66	0	0	30.0	5.00	4.00	0	0	0	0	Normal	Negative for ova or parasites.	Bl. W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
M. B.	16	10/12/20	F.	35	70%	4,032	0.87	None seen	None seen	6,000	50.3	4.66	0.66	0	37.0	6.66	0.66	0	0	0	0	Normal	10/12/20 each day after until 10/16/20	Blood W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
"	"	10/16/20	"	"	69%	4,096	0.86	None seen	None seen	6,800	50.3	4.33	0	0	35.0	5.33	3.00	0	0	0	0	Normal	Negative for ova or parasites.	Urine negative.
J. M. B.	17	6/ 2/20	M.	32	77%	4,192	0.93	None seen	None seen	6,200	60.3	9.33	0.33	0	24.6	2.33	3.00	0	0	0	0	Normal	6/2/20 each day after until 6/6/20.	Urine negative.
"	"	6/ 6/20	"	"	80%	4,256	0.95	None seen	None seen	7,000	62.0	10.00	0.66	0.33	22.6	1.33	3.00	0	0	0	0	Normal	Negative for ova or parasites.	Urine negative.
C. D.	18	7/ 5/20	F.	27	85%	4,416	0.96	None seen	None seen	7,000	65.3	8.33	0.33	0	23.6	1.00	1.33	0	0	0	0	Normal	7/5/20 each day after until 7/9/20.	Urine negative.
"	"	7/ 9/20	"	"	82%	4,448	0.93	None seen	None seen	6,000	63.6	5.66	0	0.33	26.0	2.33	2.00	0	0	0	0	Normal	Negative for ova or parasites.	Urine negative.
D. S. C.	19	5/20/20	F.	40	65%	4,000	0.81	None seen	None seen	7,000	57.3	7.33	0	0	30.0	2.00	2.33	0	0	0	1.00	Slight incr.	5/20/20 each day after until 5/24/20.	Blood W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
"	"	5/24/20	"	"	63%	4,064	0.78	None seen	None seen	7,600	61.3	8.66	0	0.33	26.3	0.66	2.66	0	0	0	0	Slight incr.	Negative for ova or parasites.	Urine negative.
G. H. T.	20	4/22/20	M.	37	72%	4,192	0.87	None seen	None seen	7,000	60.6	7.33	0	0.66	27.3	1.33	2.66	0	0	0	0	Normal	4/22/20 each day until 4/26/20.	Blood W.R. neg. Urine neg. Gum and Throat smears neg. for V. A.
"	"	4/26/20	"	"	70%	4,128	0.85	None seen	None seen	6,200	57.6	6.66	0	0.33	30.0	1.66	3.66	0	0	0	0	Normal	Negative for ova or parasites.	Urine negative.

the total number of leucocytes was practically normal, or slightly below normal in a few instances. Marked and persistent leucopenia does not seem to be a feature of this disease. As far as we were able to determine from the cases studied, there appears to be no definite relation between the degrees of lymphocytosis and the severity or chronicity of the attack. The small lymphocytes with

relatively little cytoplasm are the most common type of lymphocyte in pellagrous blood.

"In the few patients examined during the first stages of an acute attack, a tendency was noted towards a slight rise in the leucocytes to maximum normal, or a trifle beyond, but in no instance was a pronounced leucocytosis found. The differential count on these cases did

CHART III.
VARIATIONS DURING COURSE OF PELLAGRA

NAME	Case No.	Date	Sex	Age	Hb. %	R. B. C. millions	C. I.	Nucleat. Reds	Baso-philia	W. B. C. Thous.	P. M. N. %	P. M. E. %	P. M. B. %	L. lym. %	S. lym. %	L. mono. %	Trans. %	Myeloblasts %			Smudges %	Platelets	Stool Examinations	REMARKS
																		N. %	E. %	B. %				
H. B. F.	1	2/28/20	M.	56	65%	4,224	0.77	None seen	None seen	7,600	65.0	0.33	0	1.66	24.3	5.00	3.00	0	0	0	0.66	Normal	2/28/20, 2/29/20, 3/2/20, 3/3/20, 3/7/20, 4/3/20, 5/9/20 & 6/8/20. All examinations neg. for ova or parasites.	Case of chronic pellagra. When first count was made patient had no symptoms. Pt. began having symptoms on 4/3/20 and continued until after 6/7/20. Bl. W.R. neg. Gum sm. neg. for V.A. Thr. sm. neg. V.A. Urine neg. First attack pellagra.
"	"	3/ 3/20	"	"	67%	4,288	0.79	None seen	None seen	7,200	64.6	0.66	0	0.66	25.3	4.66	3.66	0	0	0	0.33	Normal		
"	"	4/ 3/20	"	"	62%	4,160	0.75	None seen	None seen	7,800	55.6	0.33	0	0.66	35.3	3.33	4.66	0	0	0	0	Normal		
"	"	5/ 7/20	"	"	65%	4,320	0.75	None seen	None seen	8,000	56.0	0.66	0.33	1.33	34.6	1.66	5.33	0	0	0	0	Normal		
"	"	6/ 7/20	"	"	65%	4,224	0.77	None seen	None seen	9,000	54.6	0	0	0.66	39.3	2.33	3.00	0	0	0	0	Normal		
M. A.	2	5/ 7/20	F.	25	72%	4,192	0.87	None seen	None seen	7,400	54.3	0	0.33	0.33	39.0	2.00	3.00	0	0	0	1.00	Normal	5/8/20, 5/9/20, 5/10/20, 5/11/20, 5/12/20, 6/5/20, 8/20/20 & 8/21/20. All examinations neg. for ova or parasites.	Patient's first blood picture was taken in attack and followed until all symptoms and signs had cleared up. Blood W. R. neg. Gum smear neg. for V. A. Throat smear neg. for V. A. Urine neg.
"	"	5/ 8/20	"	"	70%	4,096	0.87	None seen	None seen	7,200	53.6	0.33	0.33	0.66	38.3	2.66	3.33	0	0	0	0.66	Normal		
"	"	5/12/20	"	"	72%	4,128	0.87	None seen	None seen	6,800	58.6	0	0	1.00	35.6	2.00	2.66	0	0	0	0	Normal		
"	"	6/ 3/20	"	"	75%	4,224	0.89	None seen	None seen	7,200	61.6	0.66	0	0	34.3	0.66	2.66	0	0	0	0	Normal		
"	"	7/20/20	"	"	80%	4,576	0.88	None seen	None seen	7,000	64.6	8.66	0.33	0	22.6	1.66	2.00	0	0	0	0	Normal		
"	"	8/16/20	"	"	80%	4,480	0.90	None seen	None seen	7,400	67.0	7.66	1.00	0	20.3	1.66	2.33	0	0	0	0	Normal		
E. M.	3	4/10/20	F.	31	85%	4,224	1.00	None seen	None seen	6,400	54.6	0.66	0	0.66	35.3	3.33	5.33	0	0	0	0	Normal	4/11/20, 4/12/20, 4/13/20, 4/14/20, 4/15/20, 5/12/20, 6/8/20, 6/9/20, & 6/10/20. All examinations neg. for ova or parasites.	Case of chronic pellagra. First seen in attack and followed until all symptoms and signs had cleared up. Blood W. R. neg. Gum smear neg. for V. A. Throat smear neg. for V. A. Urine neg.
"	"	4/11/20	"	"	85%	4,192	1.00	None seen	None seen	6,800	53.6	0	0.33	0.33	36.6	3.00	6.00	0	0	0	0	Normal		
"	"	4/15/20	"	"	85%	4,224	1.00	None seen	None seen	7,000	61.6	0.33	0.66	0	32.3	1.66	3.33	0	0	0	0	Normal		
"	"	5/ 9/20	"	"	88%	4,704	0.92	None seen	None seen	7,200	62.6	5.33	0	0	25.0	2.66	4.33	0	0	0	0	Normal		
"	"	6/ 5/20	"	"	82%	4,672	0.89	None seen	None seen	7,000	62.6	8.66	0.66	0	20.3	1.66	6.00	0	0	0	0	Normal		
R. C.	4	3/ 3/20	M.	39	85%	4,800	0.88	None seen	None seen	8,000	65.3	6.33	0.33	0	20.0	2.00	6.00	0	0	0	0	Normal	6/5/20, 6/6/20, 6/7/20, 6/8/20, 6/9/20, 6/24/20, 7/12/20 & 9/25/20. All examinations neg. for ova or parasites.	Case seen after symptoms had cleared up and then again 3 mos. later. Blood W. R. neg. Gum smear neg. for V. A. Throat smear neg. for V. A. Urine neg.
"	"	4/ 9/20	"	"	90%	5,024	0.90	None seen	None seen	7,600	63.0	7.66	0.66	0	21.0	2.66	5.00	0	0	0	0	Normal		
"	"	5/30/20	"	"	—	—	—	None seen	None seen	—	60.6	7.00	0.33	0	23.3	2.33	6.33	0	0	0	0	Normal		
"	"	6/20/20	"	"	88%	4,960	0.89	None seen	None seen	8,000	58.3	3.33	0	0.33	30.3	2.66	5.00	0	0	0	0	Normal		
"	"	7/10/20	"	"	90%	4,864	0.92	None seen	None seen	8,600	58.3	0	0	0.33	34.3	1.66	5.66	0	0	0	0	Normal		
"	"	8/21/20	"	"	85%	4,992	0.88	None seen	None seen	7,800	59.3	0.33	0	0.33	32.6	3.33	3.66	0	0	0	0	Normal		
"	"	9/18/20	"	"	88%	4,800	0.91	None seen	None seen	8,200	59.6	3.33	0.33	0	30.0	2.00	4.66	0	0	0	0	Normal		
E. E. B.	5	4/21/20	F.	35	80%	4,224	0.95	None seen	None seen	8,000	55.0	0	0	0.66	34.6	3.33	5.33	0	0	0	1.00	Normal	4/22/20, 4/23/20, 4/24/20, 4/25/20, 4/26/20, 7/25/22, 8/27/20, 8/28/20, 8/29/20. All examinations neg. for ova or parasites.	Case seen when symptoms were severe and followed until all symptoms had cleared up. Blood W. R. neg. Gum smear neg. for V. A. Throat smear neg. for V. A. Urine neg.
"	"	4/22/20	"	"	—	—	—	None seen	None seen	—	57.0	0.33	0	0.33	32.6	3.66	6.00	0	0	0	0	Normal		
"	"	5/15/20	"	"	82%	4,576	0.99	None seen	None seen	8,400	61.0	0	0.33	0.66	31.3	2.00	4.66	0	0	0	0	Normal		
"	"	6/17/20	"	"	85%	4,480	0.96	None seen	None seen	8,200	60.0	5.00	0.33	0	27.3	2.33	5.00	0	0	0	0	Normal		
"	"	7/20/20	"	"	80%	4,544	0.88	None seen	None seen	8,000	59.6	5.66	0.66	0	25.6	3.00	5.33	0	0	0	0	Normal		
"	"	8/25/20	"	"	80%	4,640	0.89	None seen	None seen	9,000	63.0	7.33	0	0.33	21.6	1.66	6.00	0	0	0	0	Normal		

not exhibit a polynucleosis, and in only one case of acute severe pellagra were the polynuclears over 70%. A rise in polynuclears was recorded in a few recurrent chronic cases, due most likely to complicating factors.

“It has been mentioned by some workers on pellagra that the so-called large mononuclear leucocyte is relatively increased. Our own observations would not tend to substantiate this finding as a constant feature, although in a few cases a slight rise in this type of cell was noted.

“The eosinophils varied considerably, as may be seen from the table. A very moderate eosinophilia was found in occasional cases, but to state that eosinophilia is characteristic of pellagra would not be justified from a study of this analysis. The prevalence in the South of hook-worm infections, and of other forms of intestinal parasitism capable of causing an eosinophilia, is a factor to

be considered in interpreting slight fluctuations in the number of eosinophilic leucocytes.”

B. E. Giannini refers to the observation of his collaborator, Garlington, on 100 cases. He comes to these conclusions: “In all cases an anæmia was found directly in proportion to the severity of the other clinical symptoms. The white blood cells were markedly increased in acute cases, especially in robust patients. The white counts showed a leucocytosis in all cases, ranging from 10,000 to 24,000 per cubic millimetre. The differential counts showed a decrease of the eosinophils in all cases, and several hundred white cells were often counted before an eosinophile was found. There was a moderate lymphocytosis found in all cases.”

It is evident therefore that the literature abounds in contradictions in regard to the blood picture in pellagrins. In an attempt to clarify the confusion the following study was undertaken.

METHODS

The methods of examination of the blood and feces are as follows: Chart I shows 10 cases studied, all of which showed severe symptoms of pellagra. Two blood examinations were made four days apart, on each of the patients. These examinations were made as long after a meal as possible. The stool examinations were made every day for four days, the first examination being made the same day as the first blood count; the others on successive days.

Chart II shows 20 cases studied, all the patients being in the convalescent stage of the disease, when all symptoms had practically cleared up. Two blood examinations were made five days apart on each of the patients. These examinations were made as long after a meal as possible. Stool examinations were made every day for four days, the first examination being made the same day as the first blood count; the others on successive days.

Chart III shows 5 cases studied. Case I was studied first when there were no signs of the disease, and then followed into the height of the disease, when the symptoms were severe. Cases II, III and V were studied first when the symptoms of pellagra were severe, and followed until the patient was apparently well. Case IV was studied first when all symptoms of the disease had practically cleared up, and again during another attack of the disease when the symptoms were severe. The study of the blood on these five cases was made at frequent intervals, and stool examinations were made at the same time. The meal relation to the blood count was observed in this chart as in the other charts.

In all cases studied (Charts I, II and III) each examination of the blood consisted of red blood cell and white blood cell count with a differential count of 300 cells. Platelets were estimated in the smears and hæmoglobin was determined with a Sahli hæmoglobinometer. The blood was drawn from the fingers in all cases. Gum and throat smears were made and stained with methylene blue and examined for Vincent's Angina. Blood Wassermanns and spinal fluid examinations were made whenever indicated. All stools were examined by four methods. (1) *Smear method*; a small bit of the feces to be examined was mixed with distilled water on an ordinary glass slide, and then a coverglass was placed on the preparation. (2) *Brine flotation-loop method* (Kofoid and Barber)²⁹: A sample of feces was thoroughly mixed with concentrated brine. The coarse float was forced below the surface with a disc of No. 0 steel wool, and the container allowed to stand for almost an hour to give time for the eggs to ascend. The surface film was then looped off upon a slide and examined with a coverglass. (3) *Centrifugation method*: A piece of fecal material was mixed with distilled water and this mixture

strained through a sieve, which removed the larger particles. This mixture was centrifugalized and smears were made of the residue. (4) *Donaldson's iodine-eosin smear method*: The smears were prepared by rubbing out a minute bit of the feces, rolling it on a round tooth-pick in a small drop of normal salt solution, and then in an adjacent drop of iodine-eosin stain. A single cover-slip was placed on both drops and examined.

RESULTS

The findings in this series of cases seem quite definite. The principal results are as follows:

Chart I shows cases studied at the height of the disease. The hæmoglobin and red blood cells were slightly decreased in 8 of the cases; in 2 cases they were normal. The white blood count was normal in all cases except in the last count on patient No. 10, in which there was found a slight increase (10,000). This could easily be attributed to a cold which the patient had at the time. The color index was normal in all cases. The differential formula showed an increase in lymphoid elements, mainly the small lymphocytes, the highest percentage being 41.3%, the lowest being 30.3%. The polymorphonuclear elements showed a relative decrease. No bone-marrow cells were seen and smudges were those seen normally. Stool examinations showed no ova or parasites, and all other examinations were negative.

In Chart II, which shows cases studied when convalescing from the disease and practically all symptoms had disappeared, the findings were as follows: The hæmoglobin and red blood cells were slightly decreased in 18 of the cases; in 2 they could be considered as normal. The white blood counts were normal. Platelets were normal in all cases except 4, in which they were slightly increased. The color index was normal in all cases except 5, in which it was low. The differential formula showed an increase in polymorphonuclear eosinophils ranging from 4.0% to 10.0%. The polymorphonuclear neutrophils were slightly decreased in all cases except one (case 10), and there was a decrease in small lymphocytes. No bone-marrow cells were seen and smudges were not increased when counted or estimated. Stool examinations showed no ova or parasites, and all other examinations were negative.

Chart III shows cases studied throughout the disease, and also when the patients had recovered. The hæmoglobin determinations, and red blood cell counts showed a slight decrease in all cases except one (case 4), in which it remained within normal limits in all stages of the disease. The white count and the platelets remained normal throughout. The color index was normal in all cases except Case 1, in which it was low. In Case 1 the differential formula showed an increase in small lymphocytes during the period of severe symptoms. When the first count was made the patient had no symptoms of

pellagra. But as the disease developed, the small lymphocytes showed an increase. In cases 2, 3, and 5, the first counts were made at the height of the disease. There was an increase in small lymphocytes, and as the patients improved, the lymphocytes decreased and the polymorphonuclear eosinophils increased until they showed an actual increase in number reaching 8.66% in cases 2 and 3. In case 4 the first differential count was made when the patient was in the convalescent stage, practically free from symptoms. An increase in polymorphonuclear eosinophils was found. When the patient had recovered from the disease, the differential formula became normal. Finally, the patient's blood was obtained again in another attack of pellagra with severe symptoms. An increase in the small lymphocytes was found. In all 5 cases in Chart III no bone-marrow cells were found. Smudges were within normal limits. Stool examinations showed no ova or parasites and all other examinations were negative.

The main findings in all cases recorded in Charts I, II and III may be summarized as follows:—

Hæmoglobin: The hæmoglobin in most of the cases showed a slight decrease.

Red Blood Cells: The red blood cells in most of the cases showed a slight reduction.

Color Index: The color index was normal in all cases except 6. In these it was slightly low.

Leucocytes: The white blood cells in all of the cases were within normal limits except one count in Chart I, patient No. 10. Here there was a slight increase which was accounted for by the patient's having a cold.

Differential Formula: This was the most striking feature of the blood picture. In Chart I, in which the patients were all at the height of the disease and symptoms were severe, there was a definite increase in the lymphoid elements, while the polymorphonuclear cells showed a relative decrease. In Chart II, in which the patients were convalescing, there was an increase in polymorphonuclear eosinophils, ranging between 4 and 10%. In Chart III the cases were followed over a long period of time. One can see the rise and fall of the polymorphonuclear eosinophils and the lymphoid cells, this depending on the stage of the disease. The large mononuclear-transitional type of cell ranged within normal limits in Charts I, II and III, the highest percentage being 10% and the lowest 2.33%. No bone-marrow cells were seen in any of the cases studied. The smudges were so few that it was thought not necessary to count them in most of the cases. No nucleated red blood cells and no basophilia was seen in any of the cases.

Platelets: The platelets were estimated as normal in all of the cases except four, these showed a slight increase.

Stool examinations were negative in all cases for parasites or ova. The gum and throat smears were negative in all cases for Vincent's angina. In the cases where the polymorphonuclear eosinophils were increased the skin lesions had disappeared. Blood and spinal fluid Wassermanns were negative when taken.

The age incident seemed to have nothing to do with the findings. The patients in this series ranged between 21 years and 57 years.

DISCUSSION

The differential blood picture in pellagra varies with the stage of the disease, that is, at the height of the disease one finds an increase in the lymphoid elements of the blood. In the convalescent stage, when the symptoms have cleared up, one finds an increase in the polymorphonuclear eosinophils, as high as 10%. Finally, the differential formula becomes normal, when the patient has recovered. The hæmoglobin and red blood cells show a slight reduction in most cases, and one can expect to find a normal or a slightly low color index. The leucocyte count remains within normal limits and the platelets are usually normal. No nucleated red blood cells and no basophilia are found. The bone-marrow cells have not been found in any cases presented.

The eosinophilia suggest infection with intestinal parasites, but repeated stool examinations, by various methods, were negative. Apparently there is no relation between eosinophilia and the skin lesions, as the high eosinophil counts appear in cases in which the cutaneous manifestations have receded or wholly disappeared.

CONCLUSIONS

In all stages of uncomplicated pellagra the blood picture shows a secondary type of anæmia with a normal leucocyte count. The differential formula shows an increase in lymphoid elements, when the symptoms are severe. When the symptoms have subsided and the patient is in the convalescent stage, the polymorphonuclear eosinophils are increased. The platelet counts are normal.

Intestinal parasites and skin lesions play no part in the eosinophilia.

Eosinophilia augurs a favorable prognosis.

BIBLIOGRAPHY

1. Carletti, M.: Contributo all' emologica della pellagra—Padova, 1903, Tip. Prosperini.
2. Wood, T. F.: Pellagra. New York and London, 1912.
3. Hillman, O. S.: Am. J. Med. Sci., April—September, 1913. Jour. Amer. Med. Ass., Sept. 26, 1914, 43, No. 13.
4. Young, B.: Southern Med. Jour., January, 1916.
5. Fratini, G.: Revista Pella. Italiana, 1907, VII, p. 167.

6. Grigorescu, Maria C. and Galesescu, P.: Pappenheim's Folia Haematologica, May, 1904; Haematologia in pellagra. Spitalul, Bueuresci, 1903, XXIII, 682, 724.

7. Sepilli, G.: Ricerche sul sangue dei pazzi pellagrosi. Gaz. Med. Ital. Lomb., 1881, No. 43.

8. Agostini, C.: Manuale di psichiatria, Milano, 2nd ed. 1900, p. 180.

9. D'Ancona, N. and Randi, A.: Atti d. Cong. med. interprov. d. Lomb. ed Veneto, 1897; Bergamo, 1898, V, 135-140.

10. Lombroso, C.: Die Lehre von der Pellagra. Berlin, 1898.

11. Galesesco, et Slatineano: Compt. rend. Soc. de biol. Paris, 1907, LXIII, 218.

12. Lavinder, C. H.: Ann. Rep. St. Bd. of Health of S. Carolina, 1909, XXX; Virginia Med. Semi-monthly, Richmond, 1909-10, XIV, 46.

13. Castellani, A. and Chalmers, A. J.: Manual Trop. Med., London, and New York, 1919.

14. Mai, E.: Revista Pellagologica Italiana, 1912 to 1916.

15. Hyde, J. N.: Pellagra. *Diseases of the Skin*. New York 1909, 8th ed., pp. 1069-92.

16. Roberts, Stewart R.: Pellagra. C. V. Mosby Co., St. Louis, 1912.

17. Fiorini, M. e Gavini, G.: Revista critica di clinica medica. Nov. 1915, VI, 47.

18. Manson, Sir P.: Tropical Diseases. London and New York, 1907.

19. Bardin, J. C.: Amer. Jour. Insanity, July, 1913.

20. Babes and Sion: Die Pellagra. In Nothnagel, Spec. Path. und Therap., 1901, XXIV, Ht. 2.

21. Daspit, H.: New Orleans Med. and Surg. Jour., March 1909-1910, XII, 716.

22. Marie, A.: Pellagra. Trans. by Lavinder, C. H. and Babcock, J. W.: Columbia, S. C., 1910.

23. Giannini, B. E.: Kentucky Med. Jour., February, 1922, XX, 105.

24. Daniels and Newham: Laboratory Methods in Tropical Medicine.

25. Masini, M. V.: Gior. di phichiat. clin. e tech. manic. Ferrara, 1907, p. 374-381.

26. Sambon, L. W.: Brit. Med. Jour., London, 1905, II, 1272-1275; Jour. Trop. Med. and Hyg., 1910, XIII, 18, 27; also No. 19.

27. Peserico, D.: Il Morgagni, Nov., 1907. Anno XLIX, Part I.

28. Low: Edinburgh Med. Jour., 1909.

29. Kofoid, C. A. and Barber, M. A.: Jour. A. M. A. Nov. 9, 1918. Vol. 71, pp. 1557-1561.

AN INSTRUMENT FOR MEASURING DISTORTION DUE TO THE DIVERGENCE OF X-RAYS

By EBEN C. HILL

(From the Anatomical Department, The Johns Hopkins University)

The Necessity of Measuring the Distortion Due to the Divergence of X-Rays.—The many mistakes in interpretation of x-ray plates due to the distortions caused by the divergence of rays coming from a single point, have led to the development of a measuring device which enables one to control the source of error. To roentgenologists a mere presentation of the illustrations with their texts would suffice, but x-ray concerns others than roentgenologists. To understand this instrument the facts leading up to its present status of usefulness seem necessary. No longer is x-ray work confined to clinical diagnosis alone. Practically every medical school has its own equipment and most physicians have an interest in x-ray plates. To them this article is written with no perplexing scientific data and with the hope that it will cause them to realize the errors that may arise from nearness of the tube to the plate, the angle of the target rays and the importance of the position of the part radiographed.

In the early days of coil machines, blurring and magnification were quite marked. The lack of powerful equipment necessitated exposures of ten minutes or more when studying "heavy parts," and required close proximity of the tube target to the x-ray plate. At all times the effects of such very divergent rays had to be considered, but in order to develop a dependable x-ray technique, all available energy and ingenuity had to be centered on perfecting the tubes and machines. A review of

some of the difficulties with which the pioneers in this work had to contend is necessary in order to explain the causes of the distortion or enlargement of x-ray shadows. At the same time the reasons for the delay in attempting to devise methods to overcome this distortion will become apparent. It can be readily seen how previous it was to plan any campaign to correct or measure these errors, when to obtain even a fairly clear plate was most exceptional.

It was about eighteen years ago that the Holtz and coil equipments were being tested and were giving most unsatisfactory results. A plate that proved conclusively the presence of a fracture was worthy of attention. There were no spark gaps and no ampere or milliamperemeters. The great number and differences in mechanical interrupters ranged from "make and break" mercury devices to those depending upon the breaking down of sulphuric acid between insulated platinum points immersed in a jar of this acid. The electric current, when not obtained from a series of batteries which had to be continually recharged, was usually developed from a generator driven by some type of gasoline or oil engine. X-ray work depends upon a direct current; but the electrical generators and transformers of those days were primitive. So with the coil machines, reverse tubes had to be interposed between the tubes and coil lest a negative charge pass and destroy the tube. To these difficul-

ties must be added the non-dependable and expensive x-ray tube. These tubes had platinum targets with a broad focus and needed most careful attention. At times the tubes had to be baked in an oven, at others they demanded refrigeration just before using. It is no wonder that few plates were perfect or even clear enough to show the larger bones. With the plates themselves there were also difficulties too numerous to mention. The best tubes required long exposure and became heated to the breaking point. There were no Coolidge tubes to withstand the heavy prolonged currents, or reverse currents, or having their vacuum suddenly change because of the heat generated.

Many roentgenologists still use a modified gas tube for special types of work, but these new gas tubes have secondary cathodes, are easily adjustable and are broken only through carelessness. During the period of improvement of the machines and tubes, there was no method of testing the condition of the equipment except by using the hand or arm in front of a fluoroscopic screen with the rays passing through the tissues. At present there is no longer any need for such dangerous tests. Accurate and dependable meters for voltage, amperage and milliamperage, strongly built gas and Coolidge tubes, heavily leaded protective screens, and a general realization of the dangers from the careless use of the equipment have minimized the slight present day risks and make x-ray work a matter of mechanical routine. It is interesting to note that with the advent of very powerful interrupterless machines and tubes that will resist heavy strains, the former electrician, physicist, and research investigator has become a roentgenologist. The certainty of obtaining diagnostic plates makes of the roentgenologist a diagnostician and gives opportunities for the research worker.

Experiments on Divergence of X-Rays.—Studying the divergence of x-rays is simplified if considered from the standpoint of light rays. Had we an interrupterless machine and tubes of sufficient strength so that a target distance of six or eight meters could be used, the plates would show clear shadows with no distortion; the rays would be, practically speaking, parallel. But as yet a one meter target distance is as great as can be maintained without destruction of the tube or break-down in the transformer. Hence, we are compelled to compromise and, in easily penetrated objects such as the hand, we use the one meter distance and get a distortion of about eight per cent. At 65 cm. target distance this distortion averages from ten to thirty per cent, according to the distance of the object radiographed from the plate. The tube targets are now made of tungsten, but here we have chances for greater or less distortion depending upon the focal point of the cathodal stream. A very fine focal point (pin-point focus) gives little opportunity for the dispersion of secondary rays, so that we get more clearly cut shadows. But if a heavy part has to be taken, even

with the aid of a screen, there is very decided possibility of pitting or melting the focal spot because of the heavy current being concentrated on so small an area. Hence, unless we take a serious chance with an expensive tube, we must probably cut down the target distance and also use a medium or broad focus tube. The broader the focal point of the target the more diffuse are the rays given off. Consequently, we must decide upon a certain amount of divergence except in easily penetrated parts.

Another matter of great importance is the angle of incidence. Should, through carelessness, the target be held so that the rays given off are not perpendicular to the plate, we shall have very curious and confusing shadows. Rays passing obliquely through the ankle would show an apparent anterior or posterior dislocation. Often, again, errors arise because the patient changes his position. Stereoscopic plates give a depth which is important in locating structures but are of little aid in overcoming the distortion due to divergence of the rays.

The most useful device so far is the Bucky Diaphragm or the improved Potter-Bucky Diaphragm. Aside from its expense there must be complete synchronism between the length of exposure and the movement of the "grids." The first instrument required a minimum of four seconds' exposure. The later modifications have cut this time markedly, but any mechanical device needs attention and the passage of parallel metal strips perpendicular to the plate surface at times leaves "grid" marks. Then again, flash or instantaneous work is impossible. Yet this type of work is necessary in animal experimentation or in clinical studies of the heart in systole and diastole. The instrument herein described and shown in Fig. 1 is inexpensive and does aid materially, if not entirely, in controlling distortion.

The Gradual Evolution and a Description of the Measuring device.—In 1904, while being allowed to assist the late Professor Goldmann of the Frauen-Klinik of Freiburg in making mercuric injections, I noticed that a pin on the anterior surface of the body x-rayed showed marked distortion when shadowed on the plate. The exposure took somewhat longer than 20 minutes. Again, in 1910, attempts at locating a watch wheel and determining the necessity for operation gave such an enlargement of the wheel that we were greatly surprised when no radical measures were found to be necessary. Upon measurement, the wheel as shown in the x-ray shadow indicated a magnification of at least 60 per cent. Many similar occurrences where foreign bodies had entered the stomach showed the absolute necessity of some sort of measuring device. Also, there were the many fractures where the x-ray shadows showed the fragments so separated that even the most experienced surgeons felt that fascia, muscle or tendons, must be preventing a closer union.

Upon studying the records of the plates, it was noted that most of these errors occurred when the part rayed

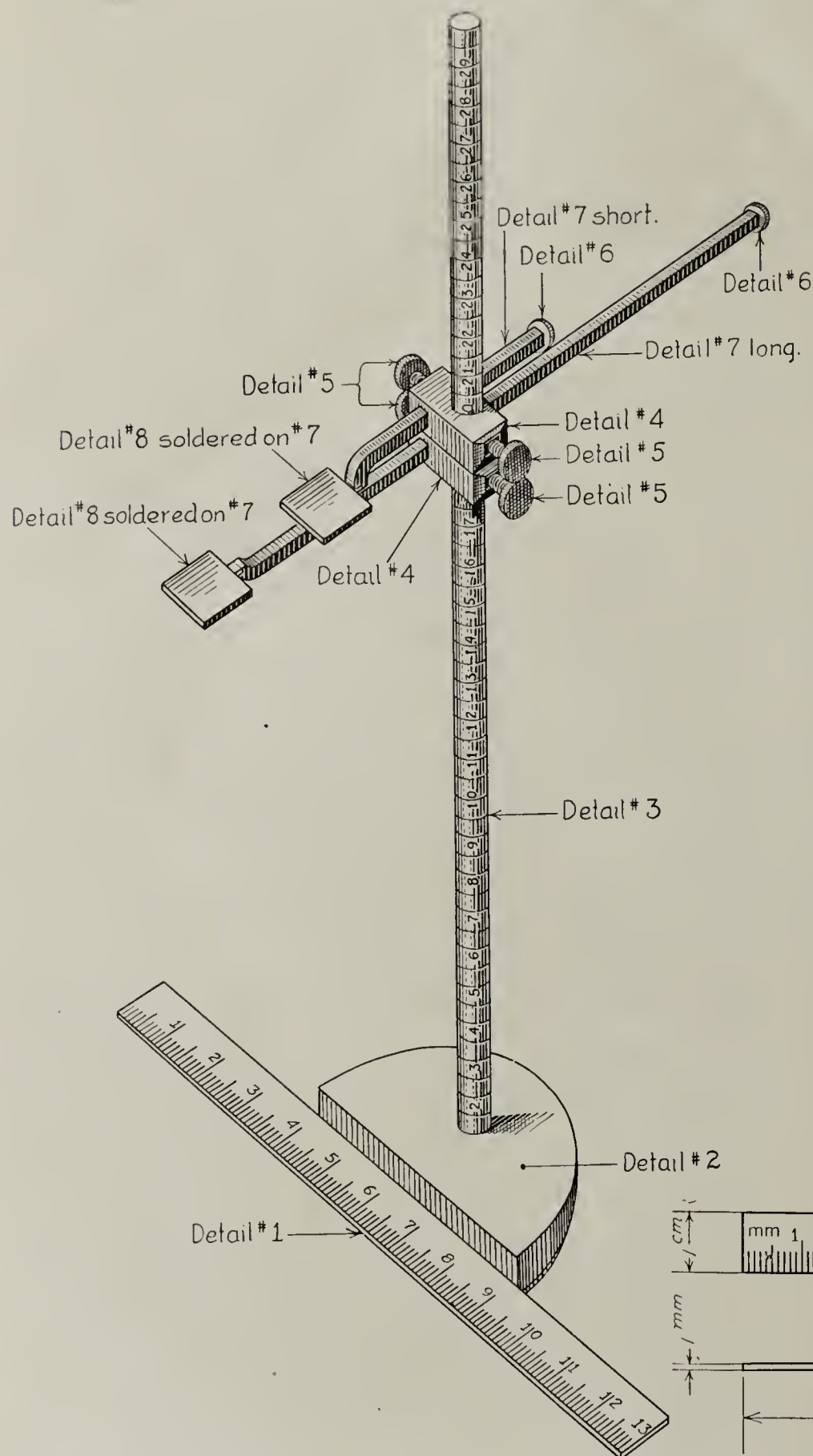


Fig. 1.—A mechanical drawing of the instrument described. This, together with Figures 2 and 3 give all working data for making this measuring device. As will be noted in context, aluminum has been successfully substituted for everything except heavy brass base.

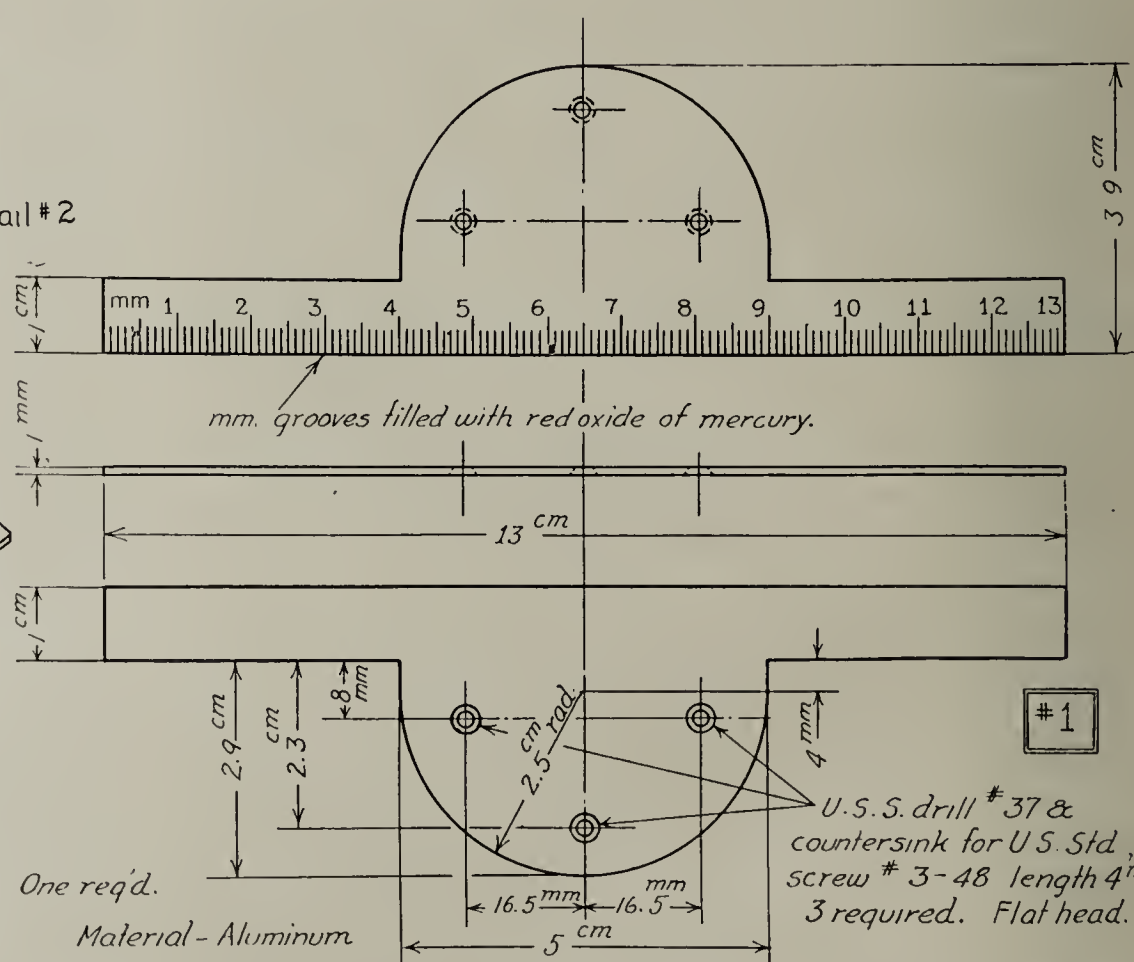


Fig. 2.—A mechanical drawing which together with Fig. 3 gives details of the instrument. As presented, it is thoroughly satisfactory, especially for very accurate measurements. Ordinarily, with instantaneous or very short exposures as in animal experimentation only the mercuric oxide millimeter markings show. Where long exposures are made, the thickness of the aluminum may be increased to 2 mm. and the depth of the markings may be cut as deep as 1 mm. Such a change would necessitate a reduction of the brass base from 9 mm. to 8 mm.

was encased in plaster of Paris or when there was great swelling. As has been noted, radiographs of heavy parts, such as thighs, or plaster-encased vertebræ, demanded, in order to give clear penetration, that the tube be quite close over the part to be studied. Also, a pin-point focus was a decided risk to the tube. An interesting test was then made by placing a twenty-five cent piece on a patient's chest and a similar coin directly on the plate. The results were most instructive when plates taken at 40, 75 and 100 cm. target distance were compared. In all of these experiments the chest coin was greatly enlarged in comparison with that on the plate. At 40 cm. target distance the enlargement was between 35% and 45%. After checking over many past observations, a serious attempt was made to devise some instrument to measure these distortions.

It was through the aid of the students in an elective x-ray course that I was able to make an instrument that in "flash" or time work, regardless of the time element, shadowed directly on the plate a millimeter scale from which direct corrections could be made with calipers. In order to determine the amount of distortion, many radiographs were made at one meter target distance. The various distances between the carpal bones were carefully measured on the plate. Then the part was dissected and direct measurements were made. At first a steel millimeter rule, 13 cm. in length, was used. So slight was the differentiation in the marks which stood for millimeters, that there was question as to its being of any practical value. The tube and machine technique was as carefully carried out as when fine silk threads show. Possibly a slightly better showing might have been made, had we used an engraved scale with more deeply cut division lines. But an instrument made on such a measuring scale as a basis would have been valueless except in very unusual instances. In the next experiment we used a very fine file and cut notches along a part of the steel measure. This measure when radiographed directly in contact with the plate covering was a success, but when moved several inches above the plate it was unsatisfactory. Any idea of attempting to use steel was abandoned when we tried to measure the distance between the styloid of the ulna and the pisiform, as accurate measurements of the wrist and especially the above given distance are most important in making a prognosis after wrist injuries. To control these measurements, the scale was raised, by using corks at the ends, to a level with the styloid process of the ulna. Plates showed clearly the tendons of the radial and ulnar sides of the hand, but the notches had become blurred and, the scale being raised to 3.75 cm. above the plate surface, the divisions on the scale had become in the radiograph 10% longer. This blurring led to the abandonment of the steel scale.

Even after this experience, it was only when an excellent A. W. Faber ruler gave a similar enlargement of the

centimeter when radiographed at the same level as in the previous experiments that a wooden base was discarded. There was no difficulty about showing millimeter markings when with an engraver's tool the wooden divisions had been deepened and mercuric-oxide had been rubbed into these grooves. But wood was not an ideal base when the edge had to be thin, and was always liable to get wet or injured.

These various experiments had shown, at least, that the accurate use of a scale depended upon that scale being in contact with the plate. Any measuring of the distortion of rays could only be done between the target and the plate. A summation of all of our experiences led to just what should have been constantly in mind, and what was explained in devoting so much space to the early difficulties in developing x-rays. In order to obtain radiographs that accurately picture the part taken, a machine and tube must be powerful enough to penetrate any portions of the body and give good contrast between tissues, at a distance of 6 or 8 m. from the target to the x-ray plate. Even with the strongest equipment and using the most effective screens, we are still far from that ideal. In olden days lawyers quite seriously asked impressive questions as to the location of "the camera" when the picture was taken. But relatively, that is what we are attempting. A camera at twenty feet is set for infinity; or for the meeting of parallel rays. We, at present, have no machines or tubes that will do flash or fractional second work at one or two meters. But we are developing instruments to do away with as many divergent rays as possible. Using a "pin-point" focus we can radiograph certain parts with little distortion from the secondary rays or from deflection of rays caused by passing through heavy tissue.

The very simple instrument I have here devised, depends for its usefulness upon the various factors earlier discussed. The device consists essentially of a metal base, in contact with the plate and holding a millimeter scale with opaque markings, with a standard from which two adjustable arms project. These carry at their ends metal plates of exactly 1 cm. square. The instrument is employed by placing the base with its scale upon the plate container and raising the arms to an appropriate distance determined by the particular part to be studied. Thus in radiographing a wrist, one metal plate is adjusted to the upper surface of the styloid. The subsequent enlargement of the square in the radiograph indicates the enlargement which occurred in these structures lying at the level of the metal square. Calipers applied to the squared area lead immediately to the determination of enlargement and this enlargement in turn may be used as the base-line of a truncated cone for the measuring of any intervening tissues. To determine whether the tissues are in any way affecting the steel measuring square, one arm may be placed above these tissues and the other

swung off at the same level but with only the second square between the target and the plate. At one-meter target distance, two instruments were used, in order to determine to what extent any differences might appear with one square in the center of a 14×14 inch plate and the other at the extreme border. The difference was negligible.

Figure 1 gives all details for a working model when used in connection with the two following mechanical drawings. To indicate the great differences in x-raying parts and foreign bodies at varying levels Fig. 4 shows measuring square "A" at a position 4 cm. above the plate while that of "B" is at 29 cm. The target distance is only 65 cm. This was done to give greater emphasis to the distortion due to divergence which in this instance was evenly "balanced," the central rays of the tube being focused upon a point midway between the two measuring squares and the base. As can be readily seen, a rectangle would result from a careless use of the tube. If the tube is not placed so that the rays will pass perpendicularly to the plate, this rectangle would probably mean that an apparent diagnosis of anterior or posterior dislocation of either the tibia or fibula was not correct. As will be noted (Fig. 1) there are two square brass arms holding at each end the square centimeter measuring plates. These plates must be parallel with the surface of the x ray plate. The measurements and dimensions of the various parts of the instrument are so carefully noted on the mechanical drawings that it is unnecessary to go into any detail about them.

The materials used and the reason for several times changing radically the device may be of interest. The first upright (Detail 3, Fig. 1) was of hexagonal brass, but had to be discarded for a round brass standard, in order to give more positions than six for the movements of the arms. The weight and dimensions of the base

were as herein shown, so that there would be stability. A second aluminum base with the scale attached is made of this metal because it is easily engraved and yet gives a firm support. It has been found that the substitution of aluminum for brass in making the measuring arms gives less chance for vibratory disturbances and practically does away with the shadows of these brass arms.

SUMMARY

1. The development of stronger, more dependable x-ray tubes and machines, together with such accessories as the intensifying screen has made possible at last a study of divergence of roentgen rays.

2. A movable diaphragm has been introduced and later modifications were made. These diaphragms remove to a certain degree the effects of secondary radiation and distortion, but there is no method for direct correction of errors.

3. The many sources of errors in diagnosis due to distorted images from the secondary and broad focus rays and to various other mechanical conditions are discussed.

4. A description, with illustrations, is given of an instrument which has been found to give accurate scientific measurements. Its usefulness is in no way dependent on the time element so necessary in the diaphragmatic devices and there is no complicated synchronism between the length of exposure and the movement of the diaphragm.

5. Calipers applied to shadows of the measuring plates and the scale quickly give the actual errors in the shadows of the part radiographed.

6. The simplicity of the instrument is self evident. By its use, in wrist measurements where radiographic determinations at a one-meter target distance as compared with dissections of the same parts showed about eight per cent error, exact control was afforded.

THE PRODUCTION OF KIDNEY LESIONS IN RATS BY DIETS DEFECTIVE ONLY IN THAT THEY CONTAINED EXCESSIVE AMOUNTS OF PROTEINS

By L. M. POLVOGT

(From the Department of Pediatrics, The Johns Hopkins University)

and E. V. MCCOLLUM and NINA SIMMONDS

(From the Department of Chemical Hygiene, School of Hygiene and Public Health, The Johns Hopkins University)

The frequent occurrence of chronic nephritis in man has not up to the present been satisfactorily explained. Even substances such as mercury may be excreted without noticeable damage to the kidney tubules, when administered in small amounts, but a severe acute nephritis

may be produced by large quantities of mercury, uranium or chromium, and the acute injury may be followed by a chronic nephritis. Recently, Newburgh¹ has attempted to show a relation between diet and the chronic nephritides. He believed that the end products of pro-

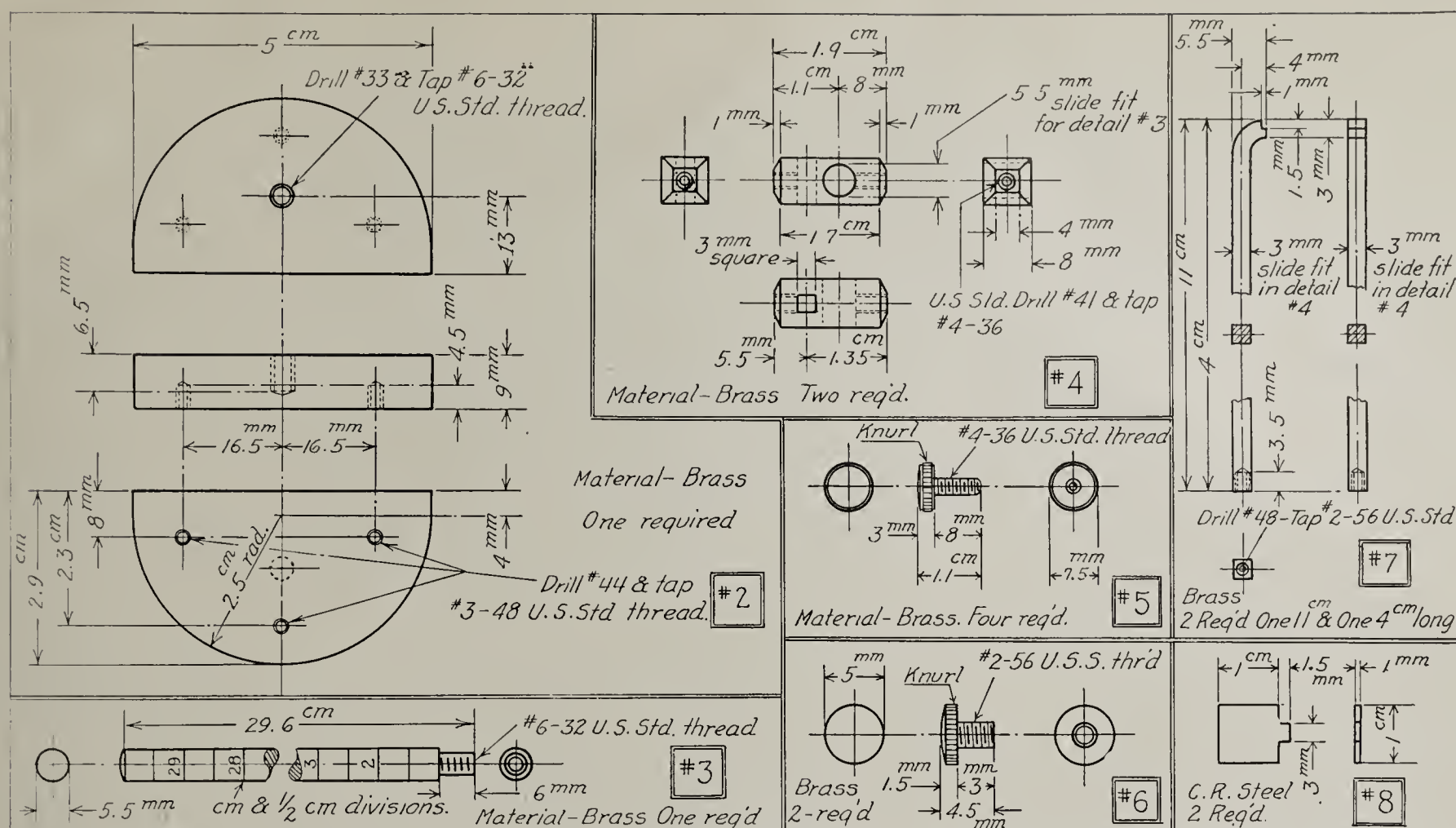


Fig. 3.—Further mechanical details of the measuring instrument as shown in Fig. 1. Detail numbering corresponds with numbers in this Fig. 1 and in Fig. 2.

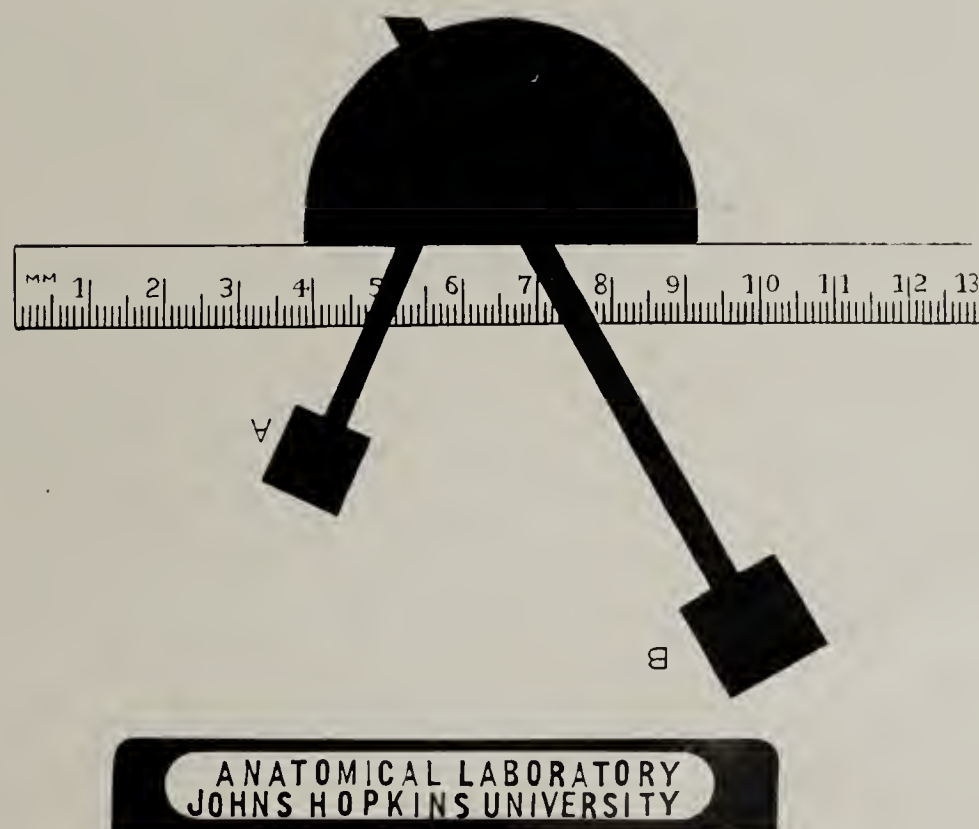


Fig. 4.—Test of instrument. Target, of medium focus, 65 cm. from X-ray plate. Steel measuring plates A, at 4 cm. and B, at 29 cm. from the plate. This target distance is the most frequently used, and the magnification of the squares is especially interesting.

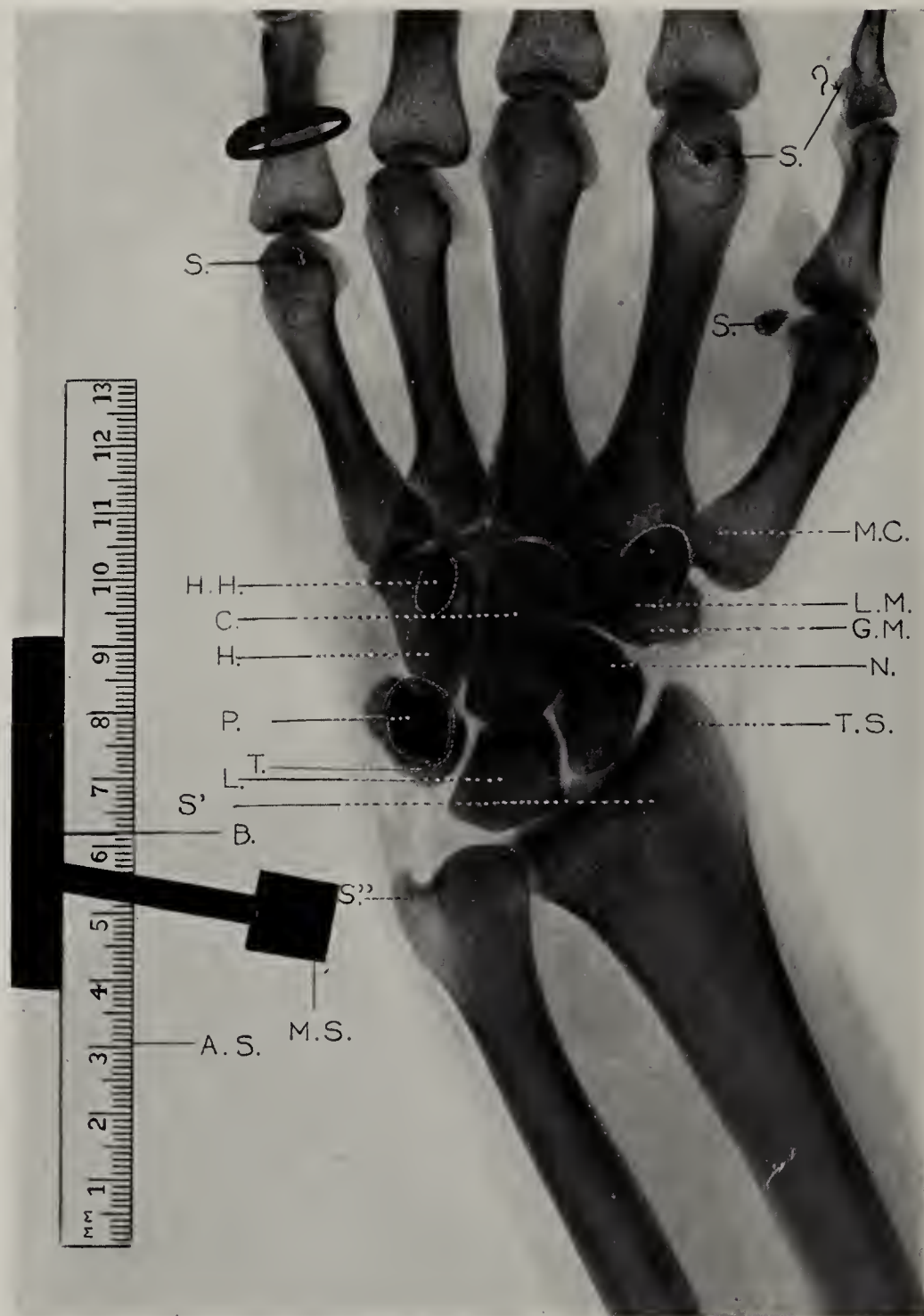


Fig. 5.—Normal hand with measuring device in position to determine distance of pisiform from styloid of the ulna. The hand is slightly to radial side. The steel marker is, of course, parallel with the plate surface and 3.75 cm. above plate at a level with the styloid. The target distance is 65 cm. Many plates at different time limits and target distances were made. This instrument, with the ever constant millimeter scale and the use of calipers, corrects in all directions shadows on the plate due to divergence of the X-rays. The position of the pisiform is of especial importance in prognosis in Colles' fractures or other wrist injuries, and it was the necessity for accurate measurements here that demanded a device similar to the one herein described. The letters on the figure beginning at the distal part of the hand are: S, sesamoid bones; L. M., lesser multangular; G. M., greater multangular; H, hammate; H. H., hook on hammate; C, capitate; P, pisiform; N, navicular; M. S., metal measuring plate; T, triquetral; L, lunate; S', styloid of radius; S'', styloid of ulna; A. S., aluminum scale; B, brass standard; M. C., metacarpal; T. S., tip of styloid of ulna. This photograph of a "soft tissue" plate has not been retouched except to reproduce what appears in the original plate.

tein metabolism might act in a manner analogous to that of the above-mentioned drugs. He felt that these substances were innocuous below a certain concentration in the blood, but that they damaged the kidneys when the amounts eliminated during a given interval exceeded the physiological limits.

He reported experiments which he interpreted as supporting this view. He fed a number of rabbits on egg-white alone. To another group of animals he gave a diet consisting of the whites of three eggs and 30 grams of cornmeal, with sufficient bran (about 25 grams) to make a stiff paste when the three ingredients were stirred together. This mixture was baked. Each rabbit was given, in addition, 25 grams of bread and some cabbage or carrots several times a week. The diet contained about 17 grams of protein, about 33 grams of carbohydrate and about 2 grams of fat. One-third of the dry weight of the diet was protein.

Still another type of diet which he used consisted of 25 c.c. of milk, 20 c.c. of water, and 20 grams of scraped carrot. This was put into a pan over a hot water bath, and when it was warm, casein and about one gram of sodium bicarbonate were added. The whole mixture was stirred and became a porous mass, which, when cool, resembled a baking-powder biscuit. Four rabbits were made to take in this way 30 grams of casein daily. Two rabbits received 15 grams of casein daily. In addition, the animals received from 25 to 30 grams of bread daily and a handful of greens about once a week.

Another group of rabbits was fed nothing but soy beans and water. His control animals were fed on hay, bread, corn-cake, cabbage, and other greens. Newburgh stated: "The only tenable explanation in the light of the results obtained with egg white and casein is one which relates the nephritis observed in each group of experiments to the common factor, namely, the high protein content of the diet."

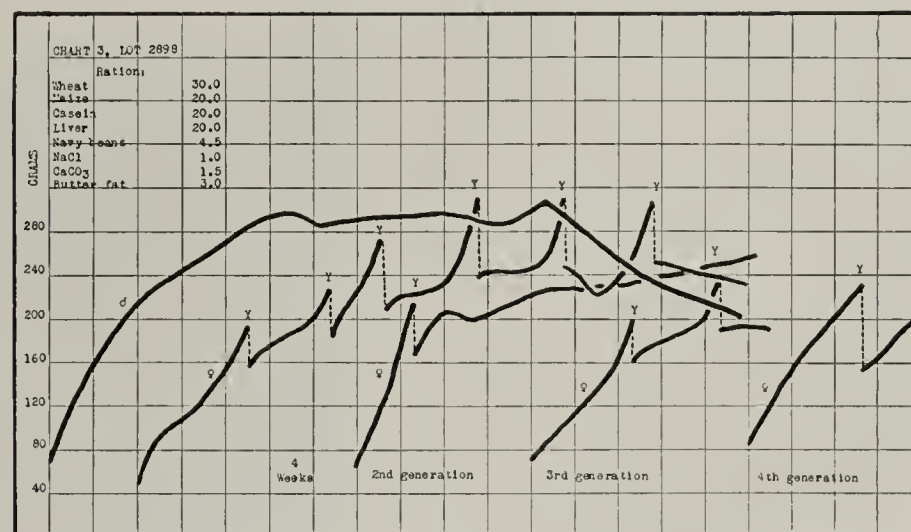
The present state of our knowledge of foods and of the nutritive requirements of animals no longer permits us to make deductions concerning the effect of a specific factor, e. g., protein, in a feeding experiment in which several other components of the diet are obviously very deficient either in amount or in quality. Thus, egg-white alone fails to nourish an animal in a satisfactory manner, not simply because of the excessive protein intake, but also because of its deficiency in certain inorganic elements and in vitamins. Egg-white alone is deficient in so many respects that the nephritis which was observed in rabbits fed on it may have been due to causes other than proteins. The same criticism applies with almost equal force to a diet of egg albumen and carrots or to an exclusive diet of soy beans, since the inorganic content of these substances combined is far from being sufficient for the satisfactory nutrition of an animal.

It is of very great importance to determine whether a diet which contains large amounts of protein but which is otherwise nearly optimal in its composition is liable to injure the kidneys. There is some reason to doubt that this is necessarily true. Certain primitive peoples have long lived essentially on strictly carnivorous diet and have eaten very large amounts of protein, and yet there is no evidence for believing that nephritis is very prevalent among them. This is also true of the carnivorous animals. We have attempted to test the effects of a high protein dietary on the rat when the diet was appropriate as regards other constituents for the maintenance of growth and well-being throughout the life of the individual or for a number of successive generations.

PLAN OF THE EXPERIMENTS

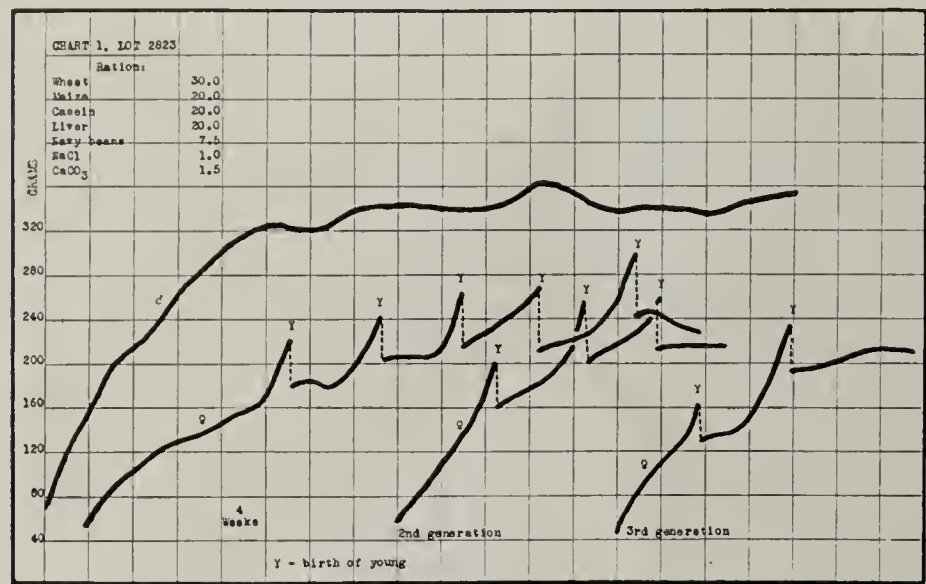
Four groups of young rats were fed diets, the protein content of which varied from 31.0 to 41.3 per cent. With the exception of the high protein content all of the diets were very well constituted, as is shown by the excellent growth records and high fertility of the animals of all groups. The observations on the kidneys were made when the rats had reached an age of about four hundred days. A number of their progeny were kept on the experimental diets until they were between two hundred and fifty and four hundred days old, when they were sacrificed and examined to see if their kidneys showed any pathological changes.

COMPOSITION OF DIETS



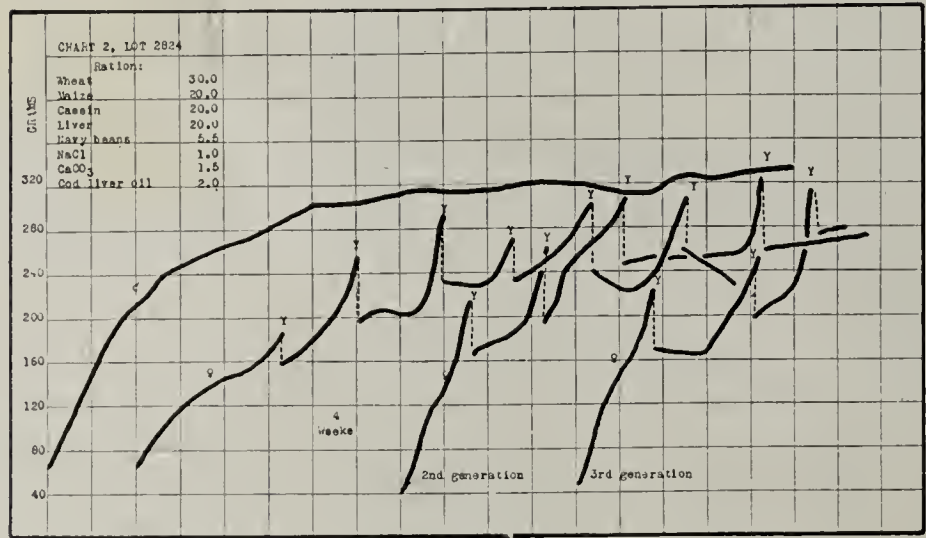
Diet 2898

Wheat	30.0	This diet contained 40.6 per cent of protein. It contained bases equivalent to 31.9 c.c. of N_{10}/HCl and acid elements equivalent to 19.7 c.c. of N_{10}/HCl .
Maize	20.0	
Casein	20.0	
Liver	20.0	
Navy beans	4.5	
NaCl	1.0	This diet was therefore potentially alkaline, and should have led to the excretion of the minimal amount of the nitrogen of the urine in the form of ammonium salts. This factor is, therefore, ruled out as a possible source of irritation of the kidney.
$CaCO_3$	1.5	
Butter fat	3.0	



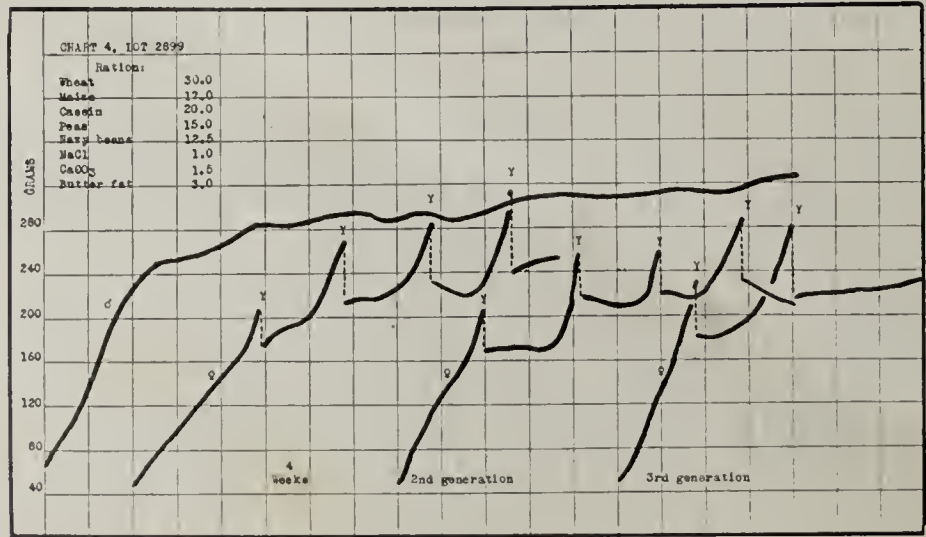
Diet 2823

Wheat	30.0	This diet contained 41.3 per cent protein. Its alkalinity was approximately the same as in diet 2898.
Maize	20.0	
Casein	20.0	
Liver	20.0	
Navy beans	7.5	
NaCl	1.0	
CaCO ₃	1.5	



Diet 2824

Wheat	30.0	40.9 per cent of protein. Alkalinity same as in 2898.
Maize	20.0	
Casein	20.0	
Liver	20.0	
Navy beans	5.5	
NaCl	1.0	
CaCO ₃	1.5	
Cod liver oil	2.0	



Diet 2899

Wheat	30.0	This diet contained 31.0 per cent of protein. It contained an excess of basic radicals over acid radicals. Total acid equivalent of 100 grams of food 28.7 c.c. N/10 HCl.
Maize	17.0	
Casein	20.0	
Peas	15.0	
Navy beans	12.5	
NaCl	1.0	
CaCO ₃	1.5	Total base equivalent of 100 grams of food 16.5 c.c. N/10 HCl.
Butter fat	3.0	

TABLE

Showing Age and Composition of the Blood with Respect to Several Constituents in Experimental and Control Rats

Lot No.	Rat No.	Generation on diet	Age at death Days	No. of days on diet	Percentage of protein in diet	Weight of rat in grams	Urea, mg. p. 100 c.c. of serum	Ser. pro., grams p. 100 c.c. ser.	Choles., mgs. p. 100 c.c. ser.	
2898A	♀ 1610	1st	522	482	40.6	248	24	7.9	112	pregnant
	♀ 1611	1st	522	482	40.6	265	28	7.8	103	
	♂ 1612	2nd	425	425	40.6	317	20	7.0	79	
	♀ 1613	2nd	425	425	40.6	220	24	8.3	76	
	♀ 1614	3rd	346	346	40.6	183	21	7.8	—	
2898B	♀ 1646	5th	129	129	40.6	155	24	6.9	58	
	♀ 1647	5th	129	129	40.6	155	21	—	—	
	♀ 1648	5th	129	129	40.6	155	28	—	52	
2824A	♂	1st	521	481	40.9	334	—	7.2	76	
	♂	1st	521	481	40.9	282	—	7.2	76	
	♀	2nd	427	427	40.9	288	—	7.3	96	
	♂	2nd	427	427	40.9	316	—	6.9	93	
2824B	♀ 1643	4th	175	175	40.9	225	22	7.2	17	
	♀ 1644	4th	175	175	40.9	198	—	7.1	—	
	♀ 1645	4th	175	175	40.9	205	18	7.0	70	
2899	♂ 1615	1st	525	483	31.0	325	26	7.1	53	pregnant
	♀ 1616	3rd	318	318	31.0	200	20	7.2	49	
	♀ 1617	2nd	404	404	31.0	281	27	6.5	75	
2823	♂ 1618	1st	525	485	41.3	355	17	8.4	—	lung lesion
	♂ 1619	1st	525	485	41.3	275	33	—	20	
	♂ 1620	3rd	164	164	41.3	250	29	7.1	42	
	♂ 1621	3rd	164	164	41.3	255	26	6.9	114	
Stock rats	♀ 1649	—	425	425	18.0	330	21	7.5	42	pregnant and lung lesion
	♂ 1650	—	425	425	18.0	280	14	6.5	41	
	♀ 1651	—	130	130	18.0	175	18	6.1	58	
	♂ 1652	—	130	130	18.0	175	—	—	—	

2898A and 2898B are animals on the same diet but are different generations.

The animals were normal to external inspection. They were very active and alert. The hair, skin, and teeth showed no deviation from those of the normal control animals. The animals varied in age from eighteen months for the first generation to five months for the fifth generation, and they had an average weight of two hundred and fifty-five grams. In only two animals were there any signs of infection found at autopsy. The first animal, a male (No. 1619), had a severe chronic infection of one lung which was studded with caseous nodules. The second animal was one of the controls, a female that was pregnant and also had a lung infection similar to the one mentioned above. Rats Nos. 1611, 1616, 1617, and

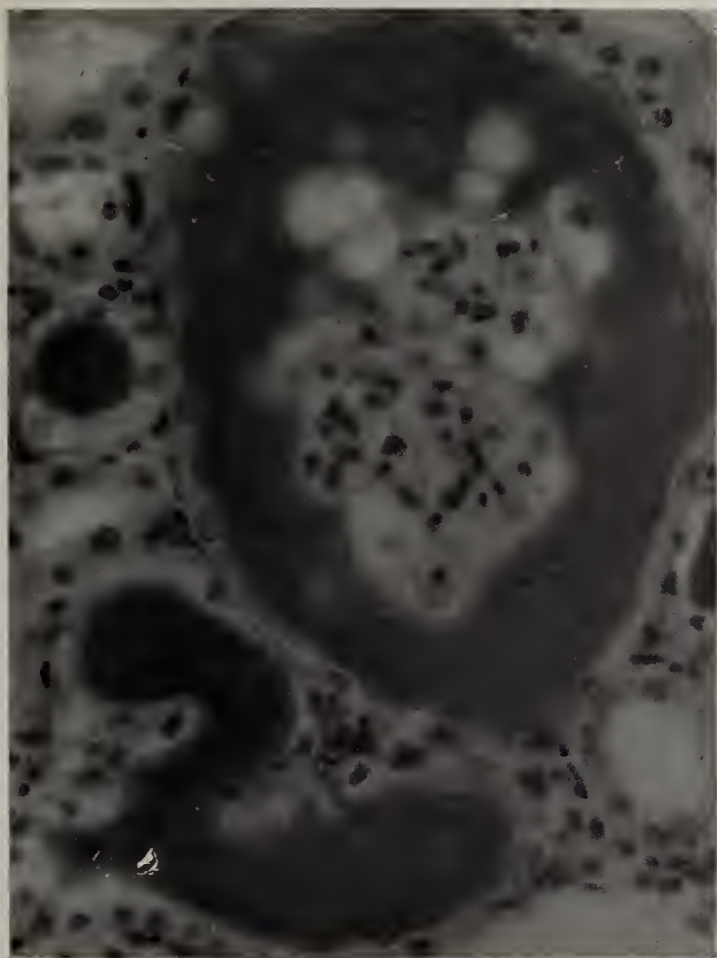


Fig. 1.

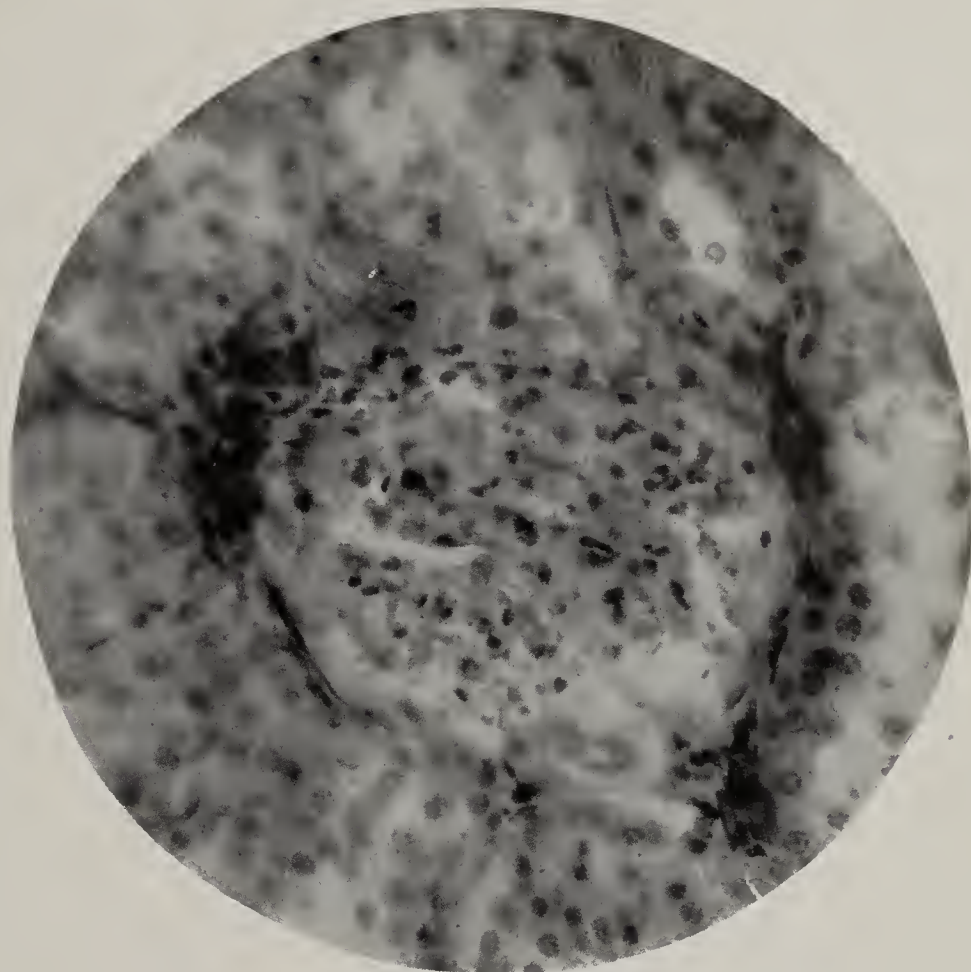


Fig. 2.

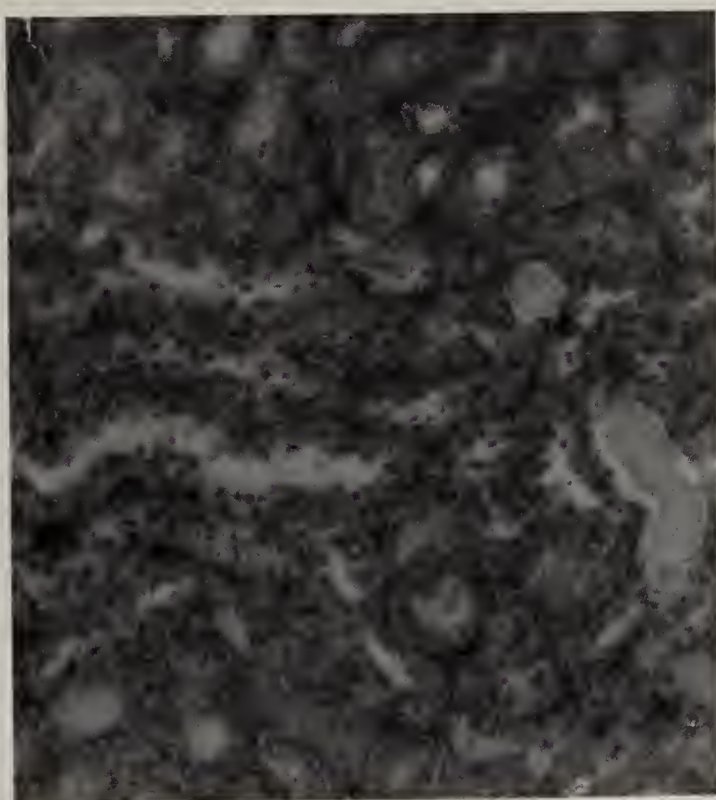


Fig. 3.

1649, were pregnant. The kidneys were the only organs in any of the other animals which were abnormal.

The kidneys of all the experimental animals showed a characteristic picture which contrasted with that observed in the kidneys of the control rats. The kidneys from animals on high protein diets were large and congested. The capsules were not adherent and could be stripped from the underlying tissues with ease. The kidneys of the control animals were smaller and firmer.

The kidneys of the animals on diet 2824A showed, without exception, similar pathological changes. They were large and apparently hemorrhagic. Microscopic examination revealed marked congestion. The capillaries were greatly distended, but there was no hemorrhage into the Bowman's capsules or the tubules. The tubular epithelium had degenerated and the lumina of the tubules were filled with cell detritus and contained hyaline casts.

All of the animals on diet 2898A showed practically the same lesions. The kidneys were very much congested. The tubules were dilated and there was some degeneration of the epithelium. The tubules were filled with cell detritus and contained hyaline casts. There was hyaline material between the glomerular tufts and the Bowman's capsules. A few gave evidence of fusion of the vessels of the tufts and the tufts were adherent to the sides of the capsules (Figs. I and II).

The kidneys of the animals on diet 2899 were large and edematous. The capsules were not adherent. The tubular epithelium was swollen and the tubules contained serum, casts and fragments of epithelium.

The animals on diet 2823 had large and congested kidneys. Microscopically, the tubules were dilated and the capillaries distended. The epithelium of many of the tubules had degenerated and the tubules contained fragments of cells, precipitated albumin, and many hyaline casts (Fig. III).

The kidneys of animals on diets 2824B and 2898B were not large but appeared hemorrhagic on gross inspection. The apparent hemorrhage was seen on microscopic examination to be due to extreme congestion. The tubules in these kidneys also contained cell detritus and albuminous exudate. There were no hyaline casts, but the clumping together of the cell detritus and exudate may probably be considered an early stage of the formation of casts.

The kidneys of the stock rats, regardless of their age or sex, were essentially normal. There was no evidence of edema or hemorrhage. The capsules were not adherent. The tubules and glomeruli were normal, and there was nowhere to be seen the desquamation and swelling of the epithelium which was present in the sections of the kidneys of rats on high protein diets.

In short, it may be said that all the rats fed the diets high in protein had lesions of the kidneys of considerable severity. This was true even of comparatively young animals of the fifth generation. On the other hand, ani-

mals of middle age from the breeding stock all had essentially normal kidneys.

The outstanding feature in all the kidneys was the formation of large numbers of hyaline casts and the intense congestion. It was surprising, considering the number of casts and the constancy with which they were found, that the glomeruli were comparatively so little involved. In general, the urea, serum protein, and cholesterol of the blood of the animals on high protein diets were within the higher limits of normal: in some cases, however, considerably higher than normal. The blood urea, serum protein, and cholesterol of the control animals all remained within the normal limits.

It is impossible, however, to avoid the conclusion that such diets as these rats were fed exert an unfavorable influence on kidney tissue. Moreover, the composition of these diets leave no room for doubt that, if protein is eaten in too high a concentration under certain conditions, the end-products of its metabolism may seriously damage the kidney.

BIBLIOGRAPHY

Newburgh, L. H.: The Production of Bright's Disease by Feeding High Protein Diets. *Archives Int. Med.*, 1919, XXIV, 359.

DESCRIPTION OF PLATES

FIG. 1.—Shows a glomerulus undergoing hyalinization and hyaline material in a convoluted tubule.

FIG. 2.—Malpighian body, showing adhesions between the glomerulus and capsule. There is a slight amount of infiltration and congestion surrounding the capsule.

FIG. 3.—To show hyaline casts in kidney tubules.

DISCUSSION OF CHARTS

The records of the rats given in Charts I to IV, together with the histological findings which we have described, furnish experimental evidence that the excretion of excessive amounts of the normal end-products of protein metabolism may result in anatomical damage to the kidneys of an omnivorous animal when all other requirements for the nutrition of this species are adequately provided for. Our diets were planned in all cases with a view to providing a mineral content which would promote good growth and prolonged well-being. The content of the diets in the several vitamins was known to be well above the minimum on which growth and health can be maintained, and were even sufficient to meet the needs of the nursing female. The only defect to which one can point as possibly existent was the excessive protein content, which varied from 31.0 to 41.3 per cent of the food mixture.

The diets, as will be seen by an inspection of these charts, induced good growth and high fertility. The infant mortality was very low, and succeeding generations did not deteriorate in a noticeable degree.

Notwithstanding the fact that the rats apparently did so well on these diets, they showed marked kidney damage without exception. The specific character of the

lesions are discussed in the text. There is apparently no cause other than the excretion of excessive amounts of the end-products of protein metabolism to which we can ascribe this renal damage.

Our data do not permit of the possible criticism that defects in the diet other than protein may have been partially or indeed solely responsible for the kidney lesions observed.

NOTES ON NEW BOOKS

Surgical Diseases of Children. By Sam. W. Kelley, M.D. (New York, E. B. Treat and Co., 1914.)

Dr. Kelley's treatise on pediatric surgery is characterized by its concise presentation of the fundamental facts belonging to this clinical subject. Brevity and clearness are a specialty of this book. Particular attention is paid to treatment, based on a wide personal experience which is so varied that the reader is much more impressed than by any mere compilation of the current literature.

In the chapter on hydrocephalus the recent investigations of Cushing, Dandy and Blackfan, Weed and McKibben, and Frazier and Peet could not be included, but in a new edition their findings cannot fail to change the therapeutic procedure recommended. In surgical tuberculosis of children, especially of the bones, heliotherapy is becoming of growing importance, and the necessity of surgical interference becomes more and more restricted to those neglected cases, which come under medical observation very late. Even when immobilization of the diseased part with a splint is being employed, heliotherapy, or the temporary substitutes for it—mercury light or X-rays—is now recommended as a powerful factor in fostering the vital resistance of the growing organism. In the chapter on empyema more stress might be laid on the re-expansion of the lung by daily blowing exercises with a Wolfe bottle or a spirometer, with the supplemental use of suction-drainage of the empyema cavity (tidal irrigation, Taylor). In the chapter on intussusception a method of resection of the intussusceptum is presented, which is undoubtedly much more complicated than the usual technic of resection of the whole involved area with a subsequent end-to-end anastomosis. In children with signs of acute intestinal occlusion, the shortest operation that will restore the normal anatomical conditions with the least possible shock to the patient is the best one.

The congenital malformations are dealt with very exhaustively along lines generally accepted. A large number of photographs and sketches, which illustrate very well a clearly presented text, make the book a reliable guide in any condition of pediatric surgery.

K. S.

The History of Medicine in its Salient Features. By WALTER LIBBY, M. A., Ph. D. \$3.00 (Boston and New York, Houghton Mifflin Co., 1922.)

This volume of some four hundred pages is a history of medicine to be read, not consulted. It is not intended to furnish

a reference book from which one may draw concise and detailed information about the work and the workers that have preceded us. It is rather to be read and enjoyed by the student, medical and lay, for its style as well as its content, and will afford real pleasure not only to the physician but to anyone interested in the cultural aspects of medicine and in good literature. The style is so excellent indeed that one suspects the writer of being outside the medical profession, and such is the case. The author has, however, partially completed a course in medicine (according to Who's Who) and has furthermore delved exhaustively into the history of science at Oxford University and on the continent. We can see no defect in the book that might be attributed to the fact that the writer is not a physician; indeed, we have the uncomfortable feeling that the literary style is benefited thereby. In any case, it cannot be too highly recommended.

J. T. K., Jr.

I Believe in God and in Evolution. By WM. W. KEEN, M. D., \$1.00 (Philadelphia and London, J. B. Lippincott Co., 1922.)

The occasion for the writing of this dissertation was an invitation extended to Dr. Keen to deliver a Commencement address at the Crozer (Baptist) Theological Seminary in 1922. It is a frank answer to the Fundamentalists, who have advocated a literal application of the Biblical story of the origin of man. The key to Dr. Keen's thought lies in the dedication of the little volume To All Sincere Seekers After Truth; Who Revere the Bible as the Word of God; Who Revere Nature as the Work of God; and Who Believe that Rightly Interpreted They Must Surely Agree. This brief statement and a few words at the end conclude the modest theological contents of the book. The author apparently feels that he is better fitted to instruct students of theology in matters pertaining to Science than in their own domain, and he devotes the best part of the address to an explanation of evolution, Darwinism and the relation of animal species. It is a book written for the layman and may be safely recommended as an excellent exposition of the broad-minded scientist's views of the compatibility of a belief in evolution and a sincere theology in the same philosophy.

J. T. K., Jr.

THE JOHNS HOPKINS HOSPITAL BULLETIN

The Hospital Bulletin contains details of hospital and dispensary practice, abstracts of papers read before the Medical Society of the Hospital, reports of lectures, and other matters of general interest in connection with the work of the Hospital. It is issued monthly. Volume XXXIV is in progress. The subscription price is \$4.00 per year.

(Foreign postage, 50 cents.) Price of cloth-bound volumes, \$5.00 each.

The Johns Hopkins Hospital Bulletins are issued monthly. They are printed by MEYER & THALHEIMER, Baltimore. Subscriptions, \$4.00 a year (foreign postage, 50 cents), may be addressed to the publishers, THE JOHNS HOPKINS PRESS, BALTIMORE; single copies will be sent by mail for fifty cents each.